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HABILITATION À DIRIGER DES RECHERCHES

Rodrigo Guabiraba

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HABILITATION À DIRIGER DES RECHERCHES

Sciences de la vie et de la Santé

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RODRIGO GUABIRABA

le 09/07/2021

JURY :
(Par ordre alphabétique)

Prénom	NOM	Grade	Établissement d'exercice
- Mme Isabelle	DIMIER-POISSON	Professeure des Universités	Université de Tours
- Mme Mariette	DUCATEZ	Directrice de Recherche	INRAE, Toulouse
- M François	MEURENS	Professeur des Universités	ONIRIS, Nantes
- M Nicolas	RITEAU	Chargé de Recherche-HDR	CNRS, Orléans
- M Bernhard	RYFFEL	Directeur de Recherche	CNRS, Orléans
- M Mustapha	SI TAHAR	Directeur de Recherche	INSERM, Tours

Je remercie tous ceux qui m'ont soutenu dans mon parcours académique.

*“There are those who think that life has nothing left to chance
A host of holy horrors to direct our aimless dance
A planet of playthings, we dance on the strings of powers we cannot perceive
The stars aren't aligned or the Gods are malign, blame is better to give than receive
You can choose a ready guide in some celestial voice
If you choose not to decide, you still have made a choice
You can choose from phantom fears and kindness that can kill
I will choose a path that's clear, I will choose Freewill
There are those who think that they were dealt a losing hand
The cards were stacked against them they weren't born in Lotus Land
All preordained, a prisoner in chains, a victim of venomous fate
Kicked in the face, you can pray for a place, in heaven's unearthly estate
You can choose a ready guide in some celestial voice
If you choose not to decide, you still have made a choice
You can choose from phantom fears and kindness that can kill
I will choose a path that's clear, I will choose Freewill
Each of us, a cell of awareness, imperfect and incomplete
Genetic blends with uncertain ends on a fortune hunt that is far too fleet
You can choose a ready guide in some celestial voice
If you choose not to decide, you still have made a choice
You can choose from phantom fears and kindness that can kill
I will choose a path that's clear, I will choose Freewill”*

Freewill (Neil Peart, 1980). In *Permanent Waves*.

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1. Curriculum Vitae

Name: Rodrigo Guabiraba **Date of birth:** 5th June, 1981.
Place of birth: Belo Horizonte, Minas Gerais – Brazil.
Work address: Infectiologie et Santé Publique (UMR 1282 ISP)
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Scientific and peer-review activities:

- i. **Researchgate:** https://www.researchgate.net/profile/Rodrigo_Guabiraba
- ii. **Publons:** <https://publons.com/author/1320127/guabiraba-r#profile>
- iii. **ORCID :** <https://orcid.org/0000-0003-4005-1753>

Education and Professional Experience:

- Feb 2000 – Dec 2007** **Bachelor, BSc (2000-2004) and Masters Degree, MSc (2005-2007) in Biological Sciences: Physiology and Pharmacology (BSc, MSc).** Federal University of Minas Gerais (UFMG, Brazil). *Supervisor:* Prof. Mauro Teixeira, PhD.
MSc dissertation title: *Role of cannabinoid receptors in inflammatory angiogenesis.*
- Feb 2007 – Dec 2010** **PhD Degree in Sciences: Immunopharmacology.**
Federal University of Minas Gerais (UFMG, Brazil) and Immunologie et Neurogénétique Expérimentales et Moléculaires INEM UMR7355 (CNRS/Université d'Orléans, France). *Supervisors:* Prof. Mauro Teixeira, PhD and Dr. Bernhard Ryffel, PhD. **Fellowship:** *CNPq, Brazil.*
PhD thesis title: *The role of iNKT cells and CC chemokines in the immunopathology of Dengue virus infection*
- Jan 2011 – Jan 2014** **Research Associate (Post-Doc)**
Institute of Infection, Immunity and Inflammation, Glasgow Biomedical Research Centre, University of Glasgow, UK. *Supervisor:* Prof. Foo Y. Liew, PhD.
Fellowship: *Wellcome Trust, UK.*
- Jan 2014 – Present:** **Chargé de Recherche - Research Scientist (Tenured)**
Infectiologie et Santé Publique (ISP UMR 1282), Institut National de la Recherche Agronomique et de l'Environnement (INRAE), Centre de Recherche Val de Loire, Nouzilly, France.

Complementary education:

Vascular Pathology (2004): Department of Physiology and Biophysics of the Biological Sciences Institute (ICB). Federal University of Minas Gerais (UFMG), Brazil.

Languages:

Portuguese (native), English (fluent, IELTS C2), French (fluent), Spanish (advanced), Italian (basic)

Grant proposals:

As the main applicant

- i. **CNPq, Brazil/CNRS, France: Sandwich PhD stage (2009-2010):** *Role of CC chemokines receptors in Dengue virus infection.* Funding : 30k euros
- ii. **Projet Jeune Chercheur, Département Santé Animale, INRAE (2015-2016):** *Implication of the platelet activating factor (PAF) system in the host immune response to Escherichia coli infection in chickens.* Funding : 10k euros.
- iii. **Projet Jeune Chercheur, Département Microbiologie et Chaîne Alimentaire, INRAE (2017-2018) :** *Defining a robust methodology to purify heterophils and identify specific markers as tools to study chicken leukocytes during avian colibacillosis.* Funding : 15k euros.
- iv. **Appels à projets « Recherche d'initiative académique », Région Centre Val de Loire (2017-Present):** **INTEGRITY** : *Intégrité de la barrière intestinale et infections opportunistes.* Funding : 205k euros.
- v. **Projet « Créativité », Département Santé Animale, INRAE (2020 – Present):** *Etude du dialogue entre les métabolites dérivés du microbiote et les récepteurs GABAérgiques de l'intestin du poulet.* Funding : 14k euros.

As a participant/partner

- i. **CNPq/INCT em Dengue, Brazil (2010-2015):** *National Institute for Science and Technology.* Funding: 5 M euros.
- ii. **CNPq/INCT em Dengue, Brazil/INSERM/Pasteur Lille (2010-2011):** *iNKT cells in human Dengue infection.* Funding : 50 k Euros.
- iii. **Universal CNPq (UFSC, Brazil) (2014-2017):** *ISG15 in infection and inflammation.* Funding: 25 k euros.
- iv. **ANIHWA ERA-NET (2015-2018):** *Understanding mucosal immunology and co- infections in the chicken to drive vaccine strategies.* Funding: 750k euros.
- v. **CAS DAR « Recherche technologique pour la compétitivité et la durabilité des filières de la production à la transformation », Ministère en charge de l'Agriculture (2017-2020):** **MEXAVI:** *Développement d'une méthodologie éprouvée permettant d'évaluer la capacité des extraits végétaux à renforcer les défenses naturelles des volailles, depuis la sélection des extraits jusqu'à la mesure de l'efficacité biologique.* Funding: 278k euros.
- vi. **CAS DAR « Recherche technologique pour la compétitivité et la durabilité des filières de la production à la transformation », Ministère en charge de l'Agriculture (2018 – Present) :** **Chick'Tip** : *Un monitoring précoce de la qualité des poussins pour une production avicole plus durable.* Funding: 298k euros.

- vii. **BBSRC, UK (2018 - Present):** *Innate immune sensing of viral DNA by avian macrophages*. Funding: 20k euros (outsourcing partner).
- viii. **FEDER, Région Centre Val de Loire (2020 - Present):** *EURO-FÉRI : Stratégie européenne de recherche pour la fédération de recherche en infectiologie*. Funding : 263k euros.
- ix. **Métaprogramme SANBA, Département Santé Animale, INRAE (2020 - Present):** *RED : Exploration de nouveaux biomarqueurs d'émotions chez la poule : le rougissement et les immunoglobulines A*. Funding : 50k euros.

Peer-reviewing activity:

Journals: PLoS One, British Journal of Pharmacology, Immunology, Viruses, Inflammation Research, Virology Journal, British Journal of Cancer, Avian Pathology, Avian Diseases, Virus Research, Nutrition Research, Scandinavian Journal of Infectious Diseases ... *among others*.

Funding bodies: *Brazilian National Agency of Research (CNPq, Brazil) (2014 – Present)*
Minas Gerais State Agency of Research (FAPEMIG, Brazil) (2015 – Present)

Editorial Board membership:

- i. BMC Veterinary Research (*Associate Editor*)
- ii. Frontiers in Immunology: Cytokines and Soluble Mediators in Immunity (*Review Editor*)
- iii. Frontiers in Pharmacology: Inflammation Pharmacology (*Review Editor*)
- iv. Frontiers in Microbiology: Infectious Diseases (*Associate Editor*)
- v. Frontiers in Pharmacology: Experimental Pharmacology and Drug Discovery (*Review Editor*)
- vi. Frontiers in Veterinary Sciences: Veterinary Infectious Diseases (*Associate Editor*)
- vii. Inflammopharmacology (*Editorial Board Member, Springer*)
- viii. International Journal of Infectious Diseases (*Editorial Board Member, Elsevier*)
- ix. International Journal of Immunopathology and Pharmacology (*Editorial Board Member, SAGE*)

Teaching activities :

Lecturer : Master 2 programme « Infectiologie Cellulaire Et Moléculaire Vaccinologie, Anticorps Thérapeutiques » - I²VB (Université de Tours, France). Topic : *Resolution of Inflammation*. Section : *Pilotage de la réponse immunitaire*.

Supervision of Students:

License Professionnelle (IUT, Université de Tours): Corentin DANNA (2016), Sofiane EL OUARIACHI (2017) and Laurine ALLIMONIER (2018).

Masters students

- i. **Amanda M. COELHO (Masters Physiology and Pharmacology, UFMG, Brazil, 2010):** *Role of Atorvastatin in experimental inflammatory angiogenesis*.

- ii. **Rafael E. MARQUES (Masters Biochemistry and Immunology, UFMG, Brazil, 2012):** *Deciphering the role of CCR5 receptor in experimental dengue infection in mice.*
- iii. **Kathleen PROUST (Master 2 ICMVAT, Université de Tours, 2014) :** *Etude de la réponse immunitaire innée lors de la colibacillose aviaire.*
- iv. **Mélanie PINAUD (Master 2 ICMVAT, Université de Tours, 2015) :** *L'Ornithokinine et ses récepteurs lors de la réponse inflammatoire induite par Escherichia coli ou son LPS en modèles cellulaires aviaires.*
- v. **Manon CHANIAL (Master 2 I²VB, Université de Tours, 2020) :** *Caractérisation des mécanismes cellulaires et moléculaires de la détection d'un acide nucléique étranger par les cellules endothéliales de poulet.*

PhD students

- i. **Geoffrey BAILLEUL (Ecole Doctorale SSBCV de l'Université de Tours, 2013 - 2016) :** *Avian defensins : A new weapon to fight enterobacteria infections ?*
Director: Anne-Christine LALMANACH (HDR) ; Co-Director : Rodrigo GUABIRABA.
- ii. **Damien GARRIDO (Ecole Doctorale SSBCV de l'Université de Tours, 2015 - 2018) :** *Impact des virus influenza aviaire faiblement pathogènes sur la réponse immune du poulet en réponse aux Escherichia coli pathogènes aviaires.*
Director: Catherine Schouler (HDR); Co-Directors: Rodrigo GUABIRABA and Sascha TRAPP.
- iii. **Vincent SAINT-MARTIN (Ecole Doctorale SSBCV de l'Université de Tours, 2020 - Present) :** *Analyse approfondie de la contribution du microbiote intestinal et de ses métabolites au développement et au fonctionnement du système immunitaire inné chez le poulet.*
Director: Pascale QUERE (HDR); Co-Director: Rodrigo GUABIRABA.

Research support activities :

- i. **Member of the *Comité d'Ethique en Expérimentation Animale Val de Loire* (CEEA VdL, 2015 – Present).**
- ii. **Participation in the organization (*pilotage*) of the *Animation Scientifique* at the UMR1282 ISP (2016 – Present):** invitation of speakers and schedule management.
- iii. **Participation in the organization (*pilotage*) of the PhD Day at the UMR1282 ISP (2016 - Present):** 1st and 2nd year PhD students present their projects and discuss with a committee (in english) in a full day dedicated to scientific exchanges.

Conferences, Oral Presentations and Workshops:

- i. **Precision cut lung slices: a novel versatile tool to examine host–pathogen interactions in the chicken lung.** 2nd ResaFlu meeting (Webinar), France - November 2020
- ii. **Precision cut lung slices: a novel versatile tool to examine host–pathogen interactions in the chicken lung.** LE STUDIUM Conference: Novel host- and microbiota-directed strategies for treating respiratory infections (Webinar), France - September 2020.

- iii. **Precision cut lung slices: a novel versatile tool to examine host–pathogen interactions in the chicken lung.** Seminars Département MICA, INRAE, France - September 2019.
- iv. **Type I IFN stimulation primes chicken macrophages to an exacerbated inflammatory phenotype mediated by IFN β following bacterial challenge.** 11th Symposium of the French Domestic Animal Immunology Network (IAD), France – March 2018.
- v. **Characterization of the Phospholipid Platelet-Activating Factor as a Mediator of Inflammation in Chickens.** Seminars MICALIS, INRAE, France – October 2016.
- vi. **IL-33 and chemotherapy induced injury.** Seminars INEM CNRS, Orléans, France - April 2014.
- vii. **Controlling inflammatory responses: Lessons from host-pathogen interaction and sterile injury.** Seminars UFMG, Brazil - November 2012.
- viii. **Dengue virus infection in mice.** Seminars Universidade Federal de Santa Catarina (UFSC), Brazil - November 2012.
- ix. **IL-22 deficiency increases IL-17 production and contributes to inflammation and tissue damage in a mouse model of severe dengue virus infection.** WIRM World Immune Regulation Meeting VI, Davos, Switzerland - March 2012.
- x. **IL-22 is involved in the control of systemic and local inflammatory responses associated to dengue virus infection in mice.** 35th Congresso da Sociedade Brasileira de Imunologia, Porto Alegre, Brazil - November 2010.
- xi. **CC chemokine receptors play different roles in the pathogenesis of dengue virus infection in mice.** 42th Congresso Brasileiro de Farmacologia e Terapêutica Experimental, Ribeirão Preto, Brazil - October 2010.
- xii. **Biology of Addictive Drugs.** Biology Week UFMG, Brazil - June 2009.
- xiii. **A Potential Major Role for the CCR5 Receptor in Mediating the Infection by Dengue Virus Serotype-2 and its Physiopathological Manifestations in Mice.** 40th Congresso Brasileiro de Farmacologia e Terapêutica Experimental, Aguas de Lindoia, Brazil - October 2008.
- xiv. **Role of cannabinoid receptors in an experimental model of inflammatory angiogenesis.** 13th International Congress of Immunology - ImmunoRio, Rio de Janeiro, Brazil - August 2007.
- xv. **Mechanisms by which the Flavonoid Dioclein Decreases Cytokines and Chemokines Levels *in vitro*.** 38th. Congresso Brasileiro de Farmacologia e Terapêutica Experimental, Ribeirão Preto, Brazil - October 2006.
- xvi. **Role of Cannabinoid Receptors in an Experimental Model of Inflammatory Angiogenesis.** 38th. Congresso Brasileiro de Farmacologia e Terapêutica Experimental, Ribeirão Preto, Brazil - October 2006.

- xvii. **Biology of Addictive Drugs.** Biology Week UFMG, Brazil - June 2006.
- xviii. **Biology of Addictive Drugs.** Biology Week UFMG, Brazil - June 2005.
- xix. **Effects of the flavonoid dioclein on cytokines and nitric oxide production by murine macrophages.** 29th Meeting of the Brazilian Society of Immunology, Ouro Preto, Brazil - October 2004.
- xx. **Antinociceptive, anti-edematogenic and anti-inflammatory effects of *Lychnophora pinaster* (arnica mineira) hydroalcoholic extract in mice.** International Symposium Nitric Oxide, Cytokines and Inflammation, Rio de Janeiro, Brazil - June 2004.
- xxi. ***Cannabis sativa* and endocannabinoids.** Seminars UEMG, Divinópolis, Brazil – June 2004.
- xxii. **Inflammation: Technical and Experimental Approach.** Biology Week UEMG, Divinópolis, Brazil - June 2004.
- xxiii. **IL-1B driven endogenous IL-10 production protects against the systemic and local acute inflammatory response following intestinal ischemia and reperfusion injury.** 34th Congresso Brasileiro de Farmacologia e Terapêutica Experimental, Aguas de Lindoia, Brazil – October 2002.

2. List of Publications

Citation report for 47 results from Web of Science Core Collection:

Results found*	47
Sum of the Times Cited	1440
Average Citations per Item	32
h-index	24

*except for Books and Book chapters

Peer-reviewed articles and reviews :

1: Souza DG, **Guabiraba R**, Pinho V, Bristow A, Poole S, Teixeira MM. IL-1-driven endogenous IL-10 production protects against the systemic and local acute inflammatory response following intestinal reperfusion injury. *J Immunol.* **2003** May 1;170(9):4759-66. PMID: 12707357.

2: Souza AL, Roffê E, Pinho V, Souza DG, Silva AF, Russo RC, **Guabiraba R**, Pereira CA, Carvalho FM, Barsante MM, Correa-Oliveira R, Fraga LA, Negrão-Correa D, Teixeira MM. Potential role of the chemokine macrophage inflammatory protein 1alpha in human and experimental schistosomiasis. *Infect Immun.* **2005** Apr;73(4):2515-23. PMID: 15784598.

3: Amaral FA, Fagundes CT, **Guabiraba R**, Vieira AT, Souza AL, Russo RC, Soares MP, Teixeira MM, Souza DG. The role of macrophage migration inhibitory factor in the cascade of events leading to reperfusion-induced inflammatory injury and lethality. *Am J Pathol.* **2007** Dec;171(6):1887-93. PMID: 18055556.

4: Russo RC, **Guabiraba R**, Garcia CC, Barcelos LS, Roffê E, Souza AL, Amaral FA, Cisalpino D, Cassali GD, Doni A, Bertini R, Teixeira MM. Role of the chemokine receptor CXCR2 in bleomycin-induced pulmonary inflammation and fibrosis. *Am J Respir Cell Mol Biol.* **2009** Apr;40(4):410-21. PMID: 18836137.

5: Barcelos LS, Coelho AM, Russo RC, **Guabiraba R**, Souza AL, Bruno-Lima G Jr, Proudfoot AE, Andrade SP, Teixeira MM. Role of the chemokines CCL3/MIP-1 alpha and CCL5/RANTES in sponge-induced inflammatory angiogenesis in mice. *Microvasc Res.* **2009** Sep;78(2):148-54. PMID: 19427874.

6: Vieira AT, Fagundes CT, Alessandri AL, Castor MG, **Guabiraba R**, Borges VO, Silveira KD, Vieira EL, Gonçalves JL, Silva TA, Deruaz M, Proudfoot AE, Sousa LP, Teixeira MM. Treatment with a novel chemokine-binding protein or eosinophil lineage-ablation protects mice from experimental colitis. *Am J Pathol.* **2009** Dec;175(6):2382-91. PMID: 19893035.

7: Victoni T, Coelho FR, Soares AL, de Freitas A, Secher T, **Guabiraba R**, Erard F, de Oliveira-Filho RM, Vargaftig BB, Lauvaux G, Kamal MA, Ryffel B, Moser R, Tavares-de-Lima W. Local and remote tissue injury upon intestinal ischemia and reperfusion depends on the TLR/MyD88 signaling pathway. *Med Microbiol Immunol.* **2010** Feb;199(1):35-42. Erratum in: *Med Microbiol Immunol.* 2010 Feb;199(1):43. PMID: 19941004.

8: **Guabiraba R**, Campanha-Rodrigues AL, Souza AL, Santiago HC, Lugnier C, Alvarez-Leite J, Lemos VS, Teixeira MM. The flavonoid dioclein reduces the production of pro-inflammatory mediators in vitro by inhibiting PDE4 activity and scavenging reactive oxygen species. *Eur J Pharmacol.* **2010** May 10;633(1-3):85-92. PMID: 20152831.

- 9:** Soares AL, Coelho FR, **Guabiraba R**, Kamal M, Vargaftig BB, Li L, Li J, Tavares-de-Lima W, Ryffel B. Tumor necrosis factor is not associated with intestinal ischemia/reperfusion-induced lung inflammation. **Shock**. **2010** Sep;34(3):306-13. PMID: 20160673.
- 10:** Russo RC, Garcia CC, Barcelos LS, Rachid MA, **Guabiraba R**, Roffê E, Souza AL, Sousa LP, Mirolo M, Doni A, Cassali GD, Pinho V, Locati M, Teixeira MM. Phosphoinositide 3-kinase γ plays a critical role in bleomycin-induced pulmonary inflammation and fibrosis in mice. **J Leukoc Biol**. **2011** Feb;89(2):269-82. PMID: 21048214.
- 11:** Garcia CC, Russo RC, **Guabiraba R**, Fagundes CT, Polidoro RB, Tavares LP, Salgado AP, Cassali GD, Sousa LP, Machado AV, Teixeira MM. Platelet-activating factor receptor plays a role in lung injury and death caused by Influenza A in mice. **PLoS Pathog**. **2010** Nov 4;6(11):e1001171. PMID: 21079759.
- 12:** Garcia CC, **Guabiraba R**, Soriani FM, Teixeira MM. The development of anti-inflammatory drugs for infectious diseases. **Discov Med**. **2010** Dec;10(55):479-88. PMID: 21189219.
- 13:** **Guabiraba R**, Marques RE, Besnard AG, Fagundes CT, Souza DG, Ryffel B, Teixeira MM. Role of the chemokine receptors CCR1, CCR2 and CCR4 in the pathogenesis of experimental dengue infection in mice. **PLoS One**. **2010** Dec 29;5(12):e15680. PMID: 21206747. ***Corresponding author**
- 14:** Souza AL, Souza PR, Pereira CA, Fernandes A, **Guabiraba R**, Russo RC, Vieira LQ, Corrêa A Jr, Teixeira MM, Negrão-Corrêa D. Experimental infection with *Schistosoma mansoni* in CCR5-deficient mice is associated with increased disease severity, as CCR5 plays a role in controlling granulomatous inflammation. **Infect Immun**. **2011** Apr;79(4):1741-9. PMID: 21263020.
- 15:** Renneson J, **Guabiraba R**, Maillet I, Marques RE, Ivanov S, Fontaine J, Paget C, Quesniaux V, Faveeuw C, Ryffel B, Teixeira MM, Trottein F. A detrimental role for invariant natural killer T cells in the pathogenesis of experimental dengue virus infection. **Am J Pathol**. **2011** Oct;179(4):1872-83. PMID: 21843496.
- 16:** **Guabiraba R**, Besnard AG, Marques RE, Maillet I, Fagundes CT, Conceição TM, Rust NM, Charreau S, Paris I, Lecron JC, Renauld JC, Quesniaux V, Da Poian AT, Arruda LB, Souza DG, Ryffel B, Teixeira MM. IL-22 modulates IL-17A production and controls inflammation and tissue damage in experimental dengue infection. **Eur J Immunol**. **2013** Jun;43(6):1529-44. PMID: 23505056. ***Corresponding author**
- 17:** **Guabiraba R**, Russo RC, Coelho AM, Ferreira MA, Lopes GA, Gomes AK, Andrade SP, Barcelos LS, Teixeira MM. Blockade of cannabinoid receptors reduces inflammation, leukocyte accumulation and neovascularization in a model of sponge-induced inflammatory angiogenesis. **Inflamm Res**. **2013** Aug;62(8):811-21. PMID: 23722450. ***Corresponding author**
- 18:** Besnard AG, Struyf S, **Guabiraba R**, Fauconnier L, Rouxel N, Proost P, Uyttenhove C, Van Snick J, Couillin I, Ryffel B. CXCL6 antibody neutralization prevents lung inflammation and fibrosis in mice in the bleomycin model. **J Leukoc Biol**. **2013** Dec;94(6):1317-23. PMID: 23975892.
- 19:** Pushparaj PN, Li D, Komai-Koma M, **Guabiraba R**, Alexander J, McSharry C, Xu D. Interleukin-33 exacerbates acute colitis via interleukin-4 in mice. **Immunology**. **2013** Sep;140(1):70-7. PMID: 23582173.
- 20:** Marques RE, **Guabiraba R**, Russo RC, Teixeira MM. Targeting CCL5 in inflammation. **Expert Opin Ther Targets**. **2013** Dec;17(12):1439-60. PMID: 24090198.
- 21:** **Guabiraba R**, Besnard AG, Menezes GB, Secher T, Jabir MS, Amaral SS, Braun H, Lima-Junior RC, Ribeiro RA, Cunha FQ, Teixeira MM, Beyaert R, Graham GJ, Liew FY. IL-33 targeting attenuates intestinal mucositis and

enhances effective tumor chemotherapy in mice. **Mucosal Immunol.** 2014 Sep;7(5):1079-93. PMID: 24424522.

***Corresponding author**

22: Guabiraba R, Ryffel B. Dengue virus infection: current concepts in immune mechanisms and lessons from murine models. **Immunology.** 2014 Feb;141(2):143-56. PMID: 24182427. ***Corresponding author**

23: Li D, Guabiraba R, Besnard AG, Komai-Koma M, Jabir MS, Zhang L, Graham GJ, Kurowska-Stolarska M, Liew FY, McSharry C, Xu D. IL-33 promotes ST2-dependent lung fibrosis by the induction of alternatively activated macrophages and innate lymphoid cells in mice. **J Allergy Clin Immunol.** 2014 Dec;134(6):1422-1432.e11. PMID: 24985397. ***Equal first-author contribution.**

24: Marques PE, Oliveira AG, Pereira RV, David BA, Gomides LF, Saraiva AM, Pires DA, Novaes JT, Patricio DO, Cisalpino D, Menezes-Garcia Z, Leevy WM, Chapman SE, Mahecha G, Marques RE, **Guabiraba R,** Martins VP, Souza DG, Mansur DS, Teixeira MM, Leite MF, Menezes GB. Hepatic DNA deposition drives drug-induced liver injury and inflammation in mice. **Hepatology.** 2015 Jan;61(1):348-60. doi: 10.1002/hep.27216. PMID: 24824608.

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3. Summary of Career Achievements

3.1. Introduction

Traditionally, disease is thought to result from an insufficient response of the host to infection, leading to increased replication of microorganisms and consequently disease. However, it is clear that the contact between a host and a microorganism is much more complex and may result in several possible interactions. There may be no interaction and this is likely the most common outcome after the contact of a higher eukaryote with microorganisms. Interaction may lead to colonization and colonization may or may not lead to infection. Infection may lead to no clinical symptoms or disease of varying degrees of severity. Induction of disease is not only the result of insufficient inflammatory and immune responses and the consequent increased microorganism replication. Disease may also be a result of excessive, uncontrolled, or misplaced inflammatory responses against the invading organism. Understanding that multiple interactions between a microorganism and its hosts are possible is not only an academic issue, but has potential practical relevance. Infection may not necessarily lead to disease and disease is not only the result of uncontrolled replication of a microorganism. Indeed, the inflammatory response triggered by certain infections is frequently the cause of tissue damage and death. During my whole career, I worked around these concepts to demonstrate that it is possible to separate mechanisms necessary for the host response to deal with infection from those that cause unwanted inflammation and drive disease. The present report will detail important aspects of my scientific career, which included the establishment of a consistent collaborative research network, extensive student's supervision, grant writing activities, and publication of relevant information in the fields of immunology, inflammation and infectious diseases of mammals and birds. I believe all these activities represent a solid base to support my request to obtain a *Habilitation à Diriger des Recherches* (HDR). By understanding mechanisms which drive disease and where/how interaction leads to disease, we may be able to devise novel therapies to alleviate suffering of animals and patients. All these possibilities were continuously discussed and explored during my prior (abroad) and recent career paths at the INRAE.

3.2. Intestinal ischemia and reperfusion injury

I started working in one of the strongest lines of research in Professor Mauro M. Teixeira's group in 2002, one year after my access to the University of Minas Gerais (UFMG), studying ischemia and reperfusion (I/R) injury in vascular beds, such as in the gut. Impaired blood flow to tissues caused by reduced or obstructed arterial inflow (ischemia) and consequent decreasing of oxygen and nutrient supply is an intrinsic condition during clinical procedures, including coronary angioplasty, vascular reconstruction, organ transplantation, and vascular diseases, such as stroke, myocardial, renal, and intestinal infarction (Gonzalez et al., 2015, Vollmar and Menger, 2011). Although reperfusion brings blood flow and oxygen back, which are essential to prevent irreversible tissue injury, it may paradoxically worsen ischemic tissue damage (Schoenberg and Beger, 1993). During reperfusion, there is excessive production of pro-inflammatory molecules by the ischemic tissue and systemic distribution of these molecules. I/R injury is responsible for up to 10% of early transplant failures and is also associated with high rates of acute and chronic rejection. Although the degree of injury may vary in different tissues, a common feature in all organs is microvascular dysfunction. The vascular injury induced by IR is a consequence of local and systemic inflammatory response and includes vascular permeability, endothelial cell activation, platelet-neutrophil interaction, complement activation, cytokine/chemokine production and imbalance between vasodilating and vasoconstricting factors (**Figure 1**) (Schoenberg and Beger, 1990, Vollmar and Menger, 2011). Understanding the role of pro and anti-inflammatory cytokines in

the events leading to tissue damage, mainly through neutrophil activation and accumulation, would eventually clarify mechanisms underlying severe intestinal I/R (i-I/R). Working with fine surgical procedure in rats, we published a research work showing that IL-1 β -driven endogenous IL-10 production protected against the systemic and local acute inflammatory response following i-I/R (**Publication 1**). As pro-inflammatory cytokines may switch ON the production of anti-inflammatory cytokines during acute and chronic inflammation, the ability of cytokine-based strategies to modulate secretion of the anti-inflammatory cytokine IL-10 is still being pursued for other inflammatory diseases (Ouyang et al., 2011, Huber et al., 2000).

In the following years, I was strongly involved in experimental design and execution of research published in 3 other articles using similar experimental models, mainly contributing to new experimental strategies or to different immunopathological analysis. Using a mouse model of i-I/R, we investigated the contribution of MIF (Macrophage migration inhibitory factor) to the injury and lethality that occurs after reperfusion of the ischemic mesenteric artery in mice (**Publication 3**). MIF can be rapidly released from intracellular stocks in leukocytes and has the capacity to modulate the production of TNF- α , an important mediator of acute inflammation (Sinitski et al., 2019, Bach et al., 2008). Leukocytes isolated from lungs of MIF-deficient mice were less activated, as assessed by their response to zymosan in a luminol-enhanced chemiluminescence assay, which was set up by myself in the research group.

This early experience with the experimental models of i-I/R led to the collaboration with Dr. Wotan Tavares de Lima (USP, Brazil) during the time I was in France with Dr. Bernhard Ryffel's group (CNRS Orléans, 2009-2010). His group was interested in the translocation of bacteria and/or bacterial products and Toll-like receptors (TLRs) activation during i-I/R, leading to acute intestinal and lung inflammation observed upon trauma. The innate immune system detects the invasion of microorganisms through toll-like receptors (TLRs), which recognize microbial components and trigger inflammatory responses. Toll-like receptors comprise a family of pattern-recognition receptors that detect conserved molecular products of microorganisms, such as LPS and lipoteichoic acid, recognized by TLR4 and TLR2, respectively. The bacterial ligands recognized by TLRs are not unique to pathogens, but rather are shared by entire classes of bacteria, and are produced therefore by commensal microorganisms as well. Toll-like receptors convert the recognition of pathogen-associated molecules in the gut into signals for antimicrobial peptide expression, barrier fortification, and proliferation of epithelial cells. Healing of injured intestinal epithelium and clearance of intramucosal bacteria require the presence of intact TLR signalling (Chen et al., 2016, Chen et al., 2008). We importantly showed that pulmonary and intestinal inflammation following i-I/R injury depends on TLR/MyD88 signalling, being related to neutrophil recruitment into the lung and the intestine, TNF- α and IL-1 β production, capillary leak and bacteraemia (**Publication 7**). This prolific collaboration led us another publication, showing that TLR2/4 signalling induces an orchestrated cytokine/chemokine response leading to local and remote pulmonary inflammation, and therefore disruption of TLR signalling would represent an alternative therapeutic target (**Publication 9**).

My research experience with the i-I/R experimental model in rats and mice represented a solid advance in developing and using animal models of inflammation, including surgery and different histopathological approaches.

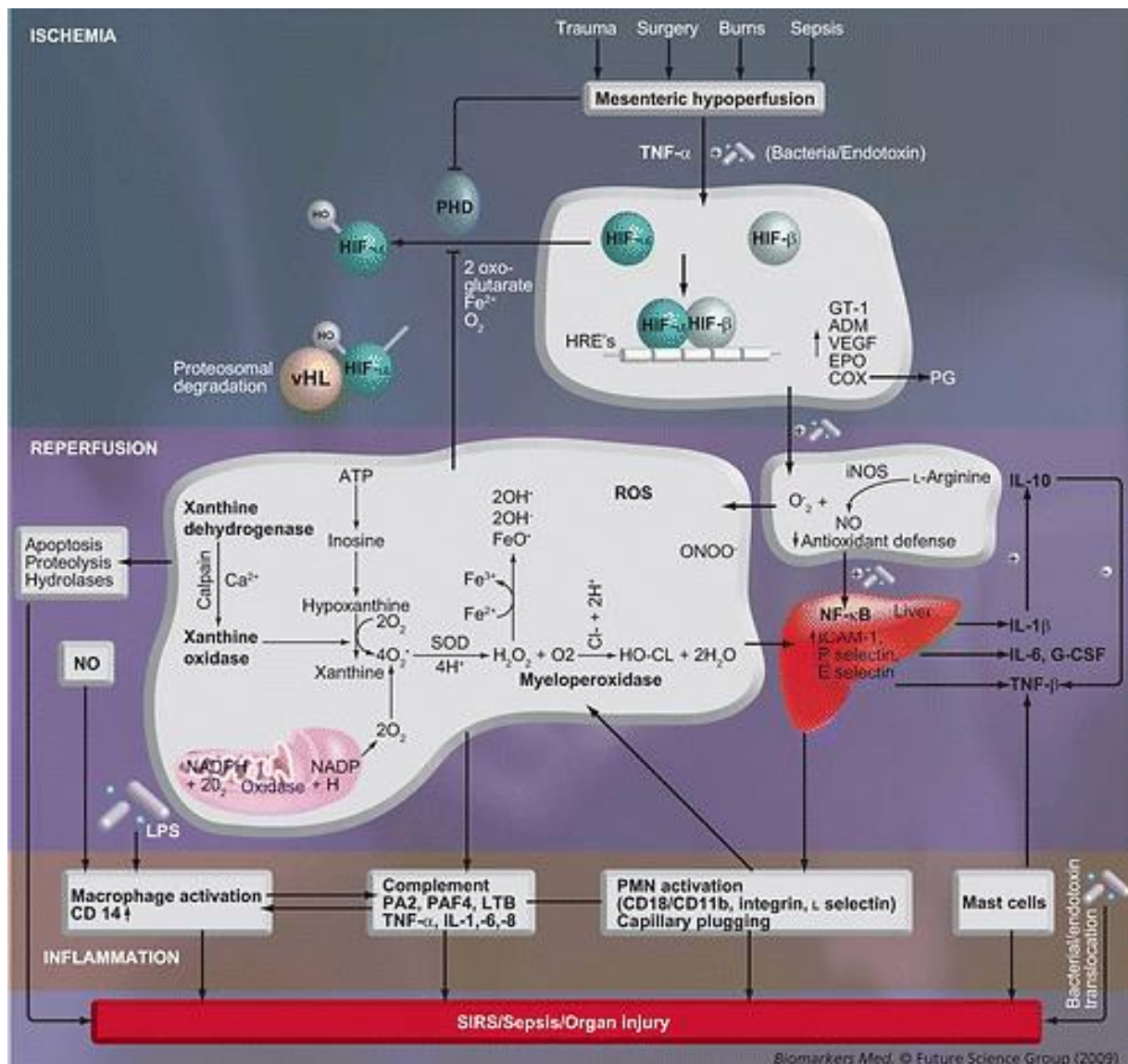


Figure 1. The physiopathology of ischemia reperfusion injury, with the role of ROS, nitric oxide and several soluble mediators (cytokines, eicosanoids, lipid mediators) in driving systemic (and local) inflammation and tissue damage (Kinross et al., 2009).

3.3. Pulmonary fibrosis

Initial studies designed to understand Interstitial Pulmonary Fibrosis (IPF) pathogenesis have primarily focused on mechanisms related to fibroplasia and deposition of extracellular matrix. To date, the real contribution of inflammation to trigger this pathology is controversial and still under debate (Barratt et al., 2018). Pulmonary fibrosis can be either caused by chronic inflammation or by a disrupted cross-talk between epithelial cells and fibroblasts, which can occur in the absence of inflammation. Some studies have related an important role for neutrophils in the inflammatory process that precedes lung fibrosis induced by the chemotherapy agent bleomycin in humans and mice (Williamson et al., 2015, Mouratis and Aidinis, 2011, Lazo et al., 1990).

We described protective effects of DF2162 (Dompé Pharmaceuticals, Italy), a long-acting antagonist of the chemokine receptor CXCR2 (important for the recruitment of neutrophils to inflamed sites), on the progression of lung fibrosis in bleomycin-instilled mice. Blockade of CXCR2 resulted in reduction of

neutrophil migration into the airways, reduction of pro-fibrogenic cytokines, amelioration of overall lung pathology and decreased proliferation, migration and capillary-like organization induced by IL-8 on endothelial cells (Russo *et al*, 2009). As an extension of this work, Russo and colleagues (2011) showed that pulmonary fibrosis and lethality induced by bleomycin are clearly attenuated in PI3K γ -deficient mice. PI3K γ is central in signalling diverse arrays of cellular functions in vertebrate immune cells, especially in neutrophils. In the absence of PI3K γ , there was decreased leukocyte influx and activation, decreased transcription of fibrogenic markers and reduced angiogenesis in lung tissue. I participated in all histopathological interpretation of the data in both projects. Being not only a problem of health concern among humans, the immunology and pathology of IPF is an important research field. In addition, the scientific collaboration with pharmaceutical companies and basic research groups brought me a whole new package of industry-related scientific language and approaches, including the *drug screening* concept.

The bleomycin-induced lung injury and the i-I/R models allowed a deep investigation of leukocyte biology and activation in target and remote organs upon acute inflammation. This concept is a valuable tool to establish new models of infection and inflammation in different vertebrates, where leukocyte influx and tissue damage will represent the main origin of worst disease outcome (**Figure 2**). Moreover, understand the kinetics of pro-inflammatory molecules and events during disease is a pivotal strategy to start developing therapeutic strategies or simply understand disease development.

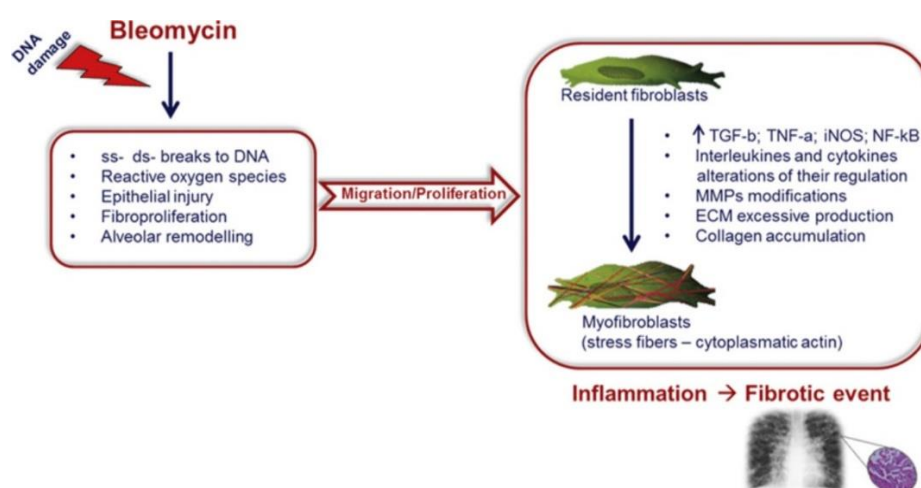


Figure 2. Bleomycin is one of the most widely used drugs for inducing lung fibrosis in animals, due to its ability to provoke a histologic lung pattern similar to that described in patients undergoing chemotherapy. This pattern is characterized by patchy parenchymal inflammation, epithelial cell injury with reactive hyperplasia, epithelial–mesenchymal transition, activation and differentiation of fibroblasts to myofibroblasts, basement membrane and alveolar epithelium injuries (Della Latta *et al.*, 2015).

3.4. Natural products and reactive oxygen species

Plant derived molecules with medicinal properties need to be fully characterized in order to serve as therapeutic agents. Among many of them, flavonoids constitute a large group of low molecular weight polyphenolic compounds derived from plants. Consumption of flavonoids in the diet has been shown to be inversely associated with morbidity and mortality from coronary heart disease around the world. In Brazil, I had the opportunity to collaborate with Professor Virginia Lemos (UFMG), an experienced physiologist working with cardiovascular diseases. At that time, Prof. Lemos was studying Dioclein, a flavonoid (flavanone family) isolated from the roots of *Dioclea grandiflora* Mart. ex Benth. She reported an important vasodilator and hypotensive effect for Dioclein, but its anti-inflammatory effects were unknown. Flavonoids have strong antioxidant properties, which may delay the onset of atherogenesis by reducing inflammatory mediators

release and decreasing thrombotic events, we decided together to study the potential anti-inflammatory properties of Dioclein. A main collaborator of Prof. Lemos, Dr. Claire Lugnier (CNRS, Strasbourg), were studying the specific inhibitory effects of Dioclein over phosphodiesterases (PDEs) isoforms. The predominant PDE that metabolizes cyclic AMP in immune cells belongs to the PDE4 (cyclic nucleotide phosphodiesterase type 4) family, and PDE4 inhibitors suppress the production of cytokines, chemokines and reactive oxygen species by macrophages. This hypothesis led our group to test the effects of Dioclein in inflammatory cells. We tested Dioclein (99% purity/HPLC) both in purified mouse neutrophils and macrophages, showing that Dioclein may serve as template for the development of novel anti-inflammatory drugs, mainly based on its ability to control the inflammatory response at different levels, which is not a common attribute for many well studied flavonoids. The double actions of Dioclein - PDE4 inhibition and strong reactive oxygen species scavenging - gave further support to the notion that drugs which act at multiple targets may be more effective at treating inflammation. Dioclein's patent was deposited and current studies are trying to characterize pharmaceutical formulations to improve its oral activity and bioavailability. An *in vivo* characterization of Dioclein in experimental rheumatoid arthritis started 2 years ago, when Prof. Teixeira and I showed that Dioclein might inhibit early neutrophil recruitment in experimental arthritis at reasonable doses (unpublished data). During this time, I got a specialization degree in **Vascular Pathology** from the Department of Physiology and Biophysics of the Biological Sciences Institute (UFMG), a course ministered by professionals in cardiovascular physiology and pharmacology in Brazil.

This study contributed to establish a link between vascular functions of a certain compound and its effects in cell populations responsible to amplify the inflammatory response. *Ex vivo* approaches are interesting tools to dissect cellular and molecular mechanisms in the interface tissue-vascular bed upon inflammation. Moreover, common mechanisms of disease, such as ROS production and cAMP/PDE4 intracellular levels, are a common link between endothelial cells in the vascular beds and infiltrating/activated leukocytes.

3.5. Inflammatory angiogenesis

Angiogenesis results from multiple signals acting on endothelial cells. Regulation of the barrier function by endothelial cells is an intricate process, requiring coordination of numerous, complex signalling pathways involved in blood vessel development and inflammatory responses. Although the link between angiogenesis and inflammation has received much attention in recent years, there has long been evidence suggesting that these are two closely related processes (**Figure 3**). These include the appearance of newly formed blood vessels in granulation tissue, and the dual functionality of angiogenic factors, i.e., they exhibit both pro-inflammatory and pro-angiogenic effects. It should be emphasized that while inflammation and angiogenesis are capable of potentiating each other, these processes are distinct and separable. Nonetheless, there is growing evidence that the angiogenesis that accompanies chronic inflammation tends to prolong and intensify the inflammatory response (Granger and Senchenkova, 2010). The model of subcutaneous sponge implants in rats was developed by Professor Silvia P. Andrade (UFMG) in 1987, when working in the Royal College of Surgeons of England, London, UK (Andrade et al., 1987). As an experimental animal system, the sponge implantation technique for the study of the inflammatory and fibrovascular components of wound healing provides an environment of defined dimensions that can be easily manipulated and examined at defined time-points and thus facilitates the kinetic tracing of different cell lineages. After being worked with the i-I/R experimental models and acquired enough experimental experience with the establishment of the pulmonary fibrosis model in Dr. Teixeira's lab, I was invited to make part of the Angiogenesis study group during my MSc (2005-2007). My first project was to identify the role of chemokines CCL3/MIP-1 α and

CCL5/RANTES (involved in the recruitment of monocytes/lymphocytes to inflammatory sites) during the development of neovascularization. The study disclosed an unexpected *in vivo* anti-angiogenic effect of the activation of CCL5/RANTES receptors (CCR1 and CCR5) during inflammatory angiogenesis in mice (**Publication 5**). Investigating chemokines as an important component of inflammatory responses associated with damage or infection was already a major topic in our group.

The interest in the cannabinoid receptors and the endocannabinoid system is still a growing field, where immune and nervous system components are closely related. Local endocannabinoid production increases in response to tissue damage during disease progression and infections. Abundant evidence demonstrates that cannabinoids have anti-inflammatory activity, which is the desired consequence notably during sterile inflammation (Witkamp, 2016, Burstein and Zurier, 2009). Sanofi-Aventis had developed Rimonabant®, an antagonist of the cannabinoid receptor CB1, to the treatment of obesity, which also presented anti-inflammatory properties in different experimental models of diseases. As a pharmacologist interested in the cell-tissue interface during inflammation, I have tested Rimonabant (CB1) and a synthetic CB2 receptor antagonist (SR144528) to study the role of the endocannabinoid system in inflammatory angiogenesis. Using immunological and physiopathological approaches, we showed that blockade of cannabinoid receptors is effective in reducing inflammatory and angiogenic responses (i.e. VEGF production, blood vessel counts). Cytokines, chemokines and endocannabinoids reduced levels may help explain this response. Partial agonism / inverse agonism activity and receptor desensitization is another explanation for the antagonist effects on cannabinoid receptors (**Publication 17**).

After working with three different and well-established experimental models of acute and chronic inflammation, dissecting cellular and molecular mechanisms involved in disease processes, I finally managed to consolidate some important features of a research carrier, which include organization and collaboration. During this time the group and myself were quite interested in basic techniques to the study of angiogenesis and tissue damage, such as morphometry, different histopathological score systems, and the pharmacology of G-protein-coupled receptors (such as cannabinoid and chemokine receptors).

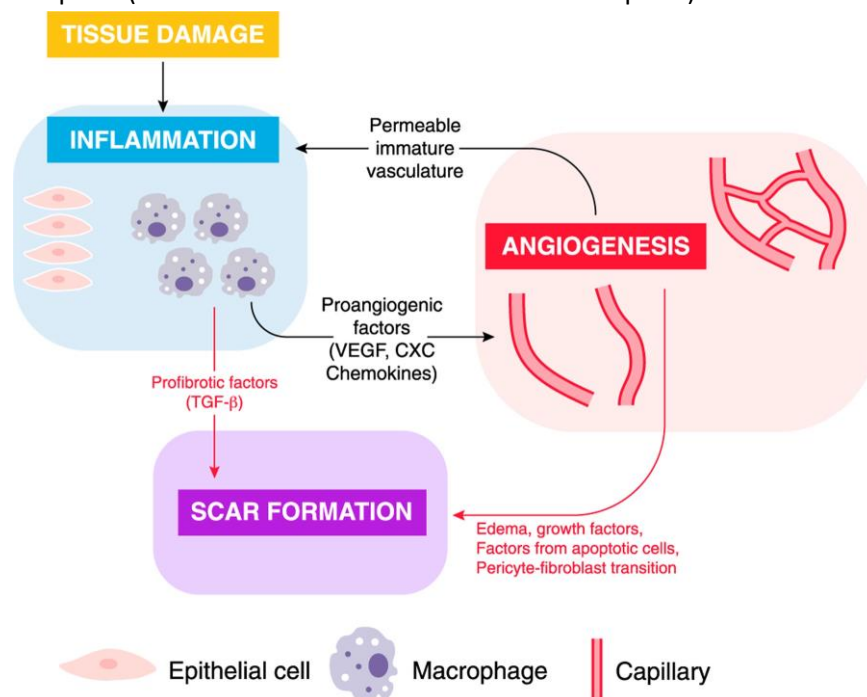


Figure 3. Macrophages, activated epithelial cells, and other inflammatory cells can release proangiogenic mediators such as VEGF and CXC chemokines, supporting robust capillary growth. In turn, the creation of the highly permeable temporary vasculature supports continued inflammation.

3.6. Chemokines and *Schistosoma mansoni* infection

Our research group in Brazil had an important collaboration with health centres focused in the study of tropical infectious diseases, topic in which Dr. Teixeira is a medical specialist (MD). Schistosomiasis is one of the most prevalent helminth infection in the world and is caused by blood flukes of the *Schistosoma* genus. In infected individuals, the granulomatous inflammation in response to egg deposition (in the liver in the case of *Schistosoma mansoni*) is the major pathological finding and accounts for exacerbated immune response, large granulomatous reactions associated with intense collagen deposition and the development of hepatosplenic schistosomiasis. Chemokines CCL2, CCL3, CCL4, CCL7, CCL11, CCL12, and chemokine receptors CCR1, CCR2, CCR3 and CCR4, have all been correlated to exacerbated experimental disease in mouse models. They are also abundant in the plasma or serum of schistosomiasis patients (Souza et al., 2006, Souza et al., 2008). Animal studies evaluating the role of chemokines in the context of schistosomiasis have used the model of granulomatous inflammation induced by the intravenous injection of eggs or beads coupled to egg antigens in immunized or non-immunized animals. The intravenous injections of beads or eggs cause pulmonary embolization followed by granuloma formation. Although this model has the advantage of synchronizing the kinetics of granuloma development, it does not take into account the host–parasite cross-talk that may have profound influence on the regulation of the inflammatory response. In addition, it does not take into account any possible host factors, in this case chemokines, in modulating the parasite metabolism. Finally, it is the liver, and not the lung, that is the most affected organ by naturally occurring granulomatous response in schistosomiasis (Souza et al., 2008).

At that time, conflicting data from different species infected with *S. mansoni* merited to be reconciled in a comprehensive model of disease pathogenesis. We therefore proposed a model in which the balance between two chemokine receptors, namely CCR1 and CCR5, exert a crucial role on the final presentation of *S. mansoni* infection. We first demonstrated a correlation between elevated concentrations of CCL3/MIP-1 α and the likelihood of presentation of severe schistosomiasis in humans, where levels of CCL3/MIP-1 α in plasma were a good marker of hepatosplenic schistosomiasis. Then, we showed that CCL3 deficiency is associated with decreased morbidity in a murine model of infection (**Publication 2**).

It was an important time for me in terms of scientific experience, which involved biosafety concepts and the use of human samples. Just after my MSc, I engaged in another project on experimental schistosomiasis, involving the chemokine receptor CCR5. This project was supported by an important grant (UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases) and accumulated evidences on the role of chemokines in the severity of schistosomiasis. Mechanistically, the absence of CCR5 caused a reduction on the recruitment of FoxP3⁺ cells (Tregs) to granulomatous lesions, increased levels of CCL3/MIP-1 α , and enhanced macrophage infiltration in liver, suggesting a modulatory role for CCR5 in the context of *S. mansoni* infection (**Publication 14**). To our knowledge, CCL5/CCR5 remains the sole chemokine/chemokine receptor pair to have a role at negatively modulating granulomatous response and to be associated with less severe disease. Consequently, the presence of key chemokines in plasma or serum, besides reflecting pathogenic mechanisms, may be used as surrogated markers of disease severity (**Figure 4**).

Techniques involving parasite recovery, *in vitro* models of infection using PBMC and mouse hepatocytes, *in vivo* animal models at different stages of disease and the study of liver physiopathology (including ultrasound investigation, and granuloma identification and isolation *in vitro*) would be expanded in the next years. These studies and the experience acquired led me to be consulted regarding the viability to carry on and help to establish the first models of viral infections arriving at Dr. Teixeira's group after 2006 (Influenza A and *Dengue virus*).

3.7. Influenza A infection and pulmonary inflammation

Influenza A viruses belong to the Orthomixoviridae family and causes annual epidemics that affect millions of people, leading to 250,000 to 500,000 deaths and 3 to 5 million severe cases annually worldwide. Strategies that aim to reduce inflammatory responses but do not affect the ability of the host to deal with Influenza A infections could reduce hospitalizations, economic losses, and morbidity. The organization of the Influenza genome provides an interesting mechanism of variation and generation of new subtypes that may cause pandemics. The antigenic shift occurs when, during a co-infection with two different subtypes, the segments are shifted to build up a new subtype. The other source of variation is the antigenic drift, which occurs after accumulated mutations that affect the antigenicity of surface glycoproteins generates epidemic strains (Cheung and Poon, 2007).

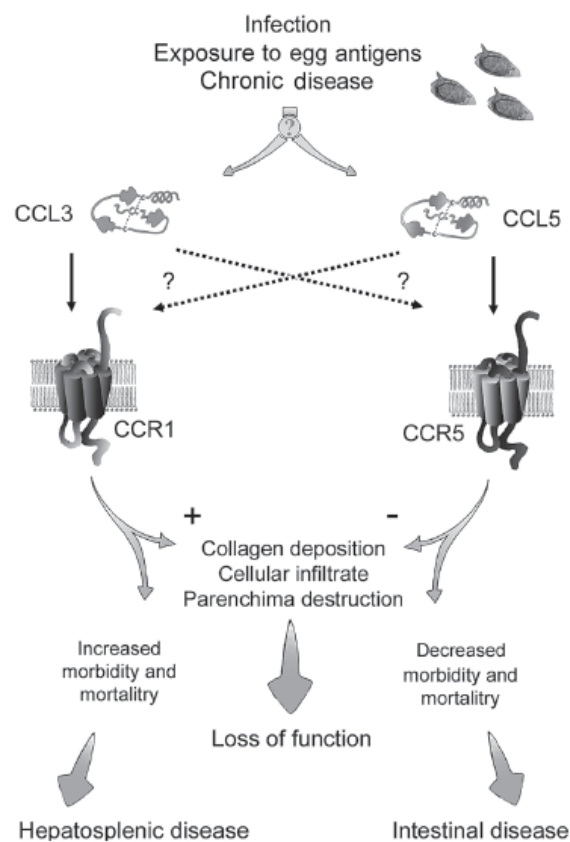


Figure 4. The majority of *Schistosoma*-infected hosts or patients, who present mild form of disease, are able to develop an adaptive immune response in which there is preponderance of CCR5 receptor activation over CCR1. On the other hand, severe forms of disease would result from unbalanced chemokine receptor activation towards an increased activation of CCR1 over CCR5. CCR1 activation would be predominantly mediated by CCL3, while CCR5 activation by CCL5. (Souza et al., 2006)

The mouse model of Influenza A infection was established in Dr. Teixeira's group in 2006 in collaboration with Prof. Ricardo T. Gazzinelli (UFMG, FIOCRUZ, Brazil), a recognised immunologist and parasitologist. Our laboratory was seeking to identify the role of Platelet Activating Factor (PAF) in viral infections. Many cell types, especially leukocytes, are able to produce PAF. PAF acts on a single receptor (PAFR) expressed in the plasma membrane or the outer leaflet of the nucleus of leukocytes, platelets, and endothelial cells (Marrache et al., 2002). By activating PAFR, PAF mediates leukocyte transmigration and activation, changes in vascular permeability, hypotension, production of various cytokines, and pulmonary damage

(Montrucchio et al., 2000). Briefly, our studies clearly showed that PAF-receptor-mediated inflammatory events that follow Influenza A virus infection are important for disease pathogenesis and lethality (**Publication 11**). Protection in PAFR-deficient mice was associated with decreased infiltration of neutrophils and macrophages into the airways and decreased lung damage (**Figure 5**). Importantly, PAFR deficiency tended to enhance the ability of the murine host to deal with the virus and antibody and adaptive responses were maintained. Treatment with PAFR antagonists starting 3 days after infection also protected against Influenza A morbidity and lethality.

The Influenza A and PAF project was conducted and designed with the support of virologists from Prof. Ricardo Gazzinelli's group (UFMG, FIOCRUZ). This collaboration was essential to solidify my interest in host-pathogen interaction in the pulmonary inflammation group. I took part in all *in vitro* study design. During the *in vivo* experiments, I worked in flow cytometry and histopathological analysis. The choice of compounds for PAF-receptor blocking and preliminary tests to identify its efficacy was conducted by the first 3 authors (including me). The model is still a valuable tool to depict the role of inflammatory mediators in tissue injury and viral clearance. At the same time, Dr. Teixeira's group was working with PAF and the new model of dengue virus infection in mice. Based on my experience at that time, I decided to contribute to the investigation of Dengue shock syndrome and its mechanisms, with a deep focus in hepatocyte degeneration and leukocyte trafficking to the liver, cytokine storm and leukocyte populations involved in the acute response to the primary infection. This choice would finally define my scientific profile up to the present moment.

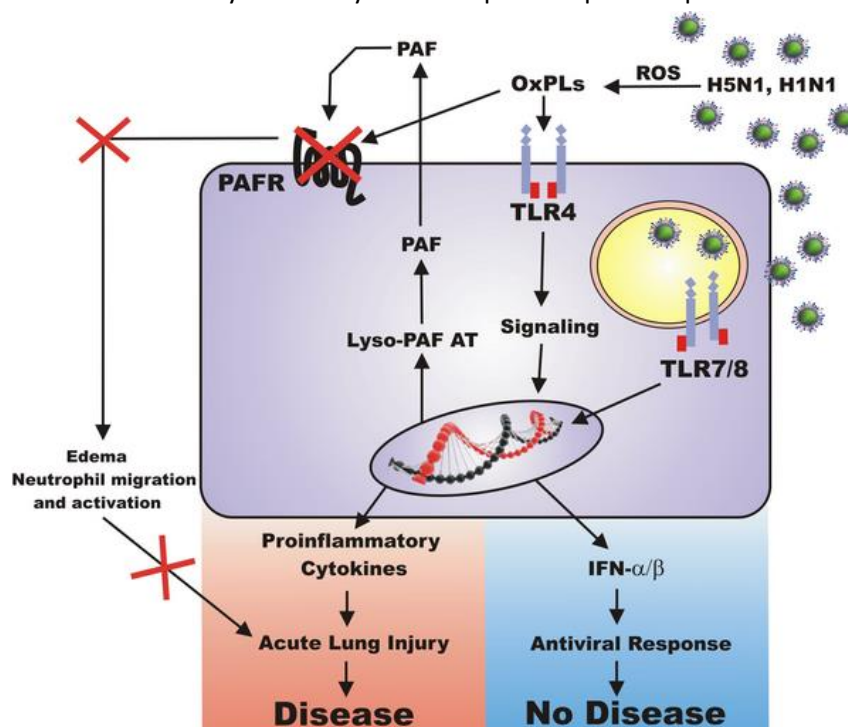


Figure 5. The role of PAF and PAFR in the context of Influenza A infection. TLR7/8 activation promotes the transcription of Lyso-PAF acetyltransferase (Lyso-PAF AT) that is responsible for PAF synthesis. PAF by binding and activating the PAFR induces neutrophil activation and migration to the airways and pulmonary edema. Influenza infection also leads to the generation of reactive oxygen species which oxidize phospholipids like LDL, generating oxidized phospholipids (OxPL). OxPL is recognized by TLR4 and activates intracellular signaling that triggers proinflammatory cytokines synthesis and ALI. OxPLs, known as PAF-like lipids, are also recognized by PAFR. Red "X" represents places of intervention through the blockade of PAFR during Influenza A infection. (**Publication 12**)

3.8. Dengue virus infection

Dengue fever and dengue shock syndrome (DF/DSS) are mosquito-borne diseases caused by 1 of 4 serotypes of *Dengue virus* (DENV 1–4). There are an estimated 50–100 million cases of dengue fever and 20,000 deaths annually, mostly in tropical and subtropical regions of the world. DSS is defined as fever with haemorrhagic manifestations, thrombocytopenia, hemoconcentration or other signs of plasma leakage, and cytokine "storm". Elevated levels of cytokines and chemokines appear to be correlated with markers of severe disease, including hepatic dysfunction, hypotension, thrombocytopenia, and hemorrhagic shock (Singh et al., 2020, Lee et al., 2016, Dejnirattisai et al., 2008, Fink et al., 2006, Bethell et al., 1998). However, mechanisms driving this massive cytokine production, a phenomenon that also occurs in bacterial sepsis and other shock related syndromes, are not known. There are no drugs approved for the treatment of dengue infection and vaccination has shown (to date) limited efficacy (de Silva and Harris, 2018). Dr. George Ignatyev (The State Research Center of Virology and Biotechnology VECTOR, also known as the Vector Institute, Russia) started his collaboration with Brazil by donating 2 mouse-adapted DENV strains (serotypes DENV-2 and 3). The nucleic acid sequence of a portion for E and NS1 genes of the mouse-adapted DENV-2 strain P23085 has been deposited previously at GenBank under accession No. AY927231.1. The partial sequence showed 98% identity with the corresponding region of other human DENV serotype 2 isolate. Different characterizations, including *in vivo* and *in vitro* neutralization and transmission electron microscopy images in purified batches, were performed. I led the characterization of the *in vitro* immune responses against our virus (mainly DENV-2), using HepG2 cells, macrophages (BMDM) and dendritic cells (BMDC). We also characterized chemokine and cytokine production profile, cytotoxicity, patterns of viral replication and virus identity (PCR). More important, we could block viral adhesion by mechanisms already described in the literature (Heparan sulphate or mannose receptor blocking, silencing or transfection). NS3 staining was also confirmed by Immunohistochemistry in liver slides, a specific technique established by myself in the group.

Recent clinical studies in endemic areas described a correlation between dengue disease outcome and blood levels of CC chemokines (Sierra et al., 2014, Islam et al., 2019), my main subject in the last years. I started studying the role of the CCR5 receptor in experimental DENV-2 infection, an important receptor involved in HIV cell entry and in the trafficking of leukocytes during *West nile virus* infection (another relevant mosquito-borne arbovirosis caused by a flavivirus). Using *in vitro* and *in vivo* strategies, we showed that mice treated with the CCR5 antagonist met-RANTES are extremely protected from disease. The same was observed in CCR5-deficient mice. We showed *in vitro* that this protection is related to viral entry (immunofluorescence and two-photon microscopy), receptor signalling (ERK, JNK) and replication, which delay the disease onset and confer an optimal viral clearance (**Publication 27**). These findings relate to the well-established association of CCR5 and flavivirus infections in an unexpected way: as a host factor that contributes to viral replication, rather than as a key molecule driving protective immune responses such as observed in West Nile virus, tick-borne encephalitis virus and Japanese encephalitis virus infections (Grygorczuk et al., 2016, Lim and Murphy, 2011, Diamond and Klein, 2006). However, diseases caused by these viruses are characterized by encephalitis, in contrast to the haemorrhagic fever and shock caused by DENV, with rare exceptions. Hence, these diseases must have pathogenic mechanisms relying on different roles for CCR5 and its ligands during infection. We got a support from Pfizer (UK) to test Maraviroc and other CCR5 antagonists in our model before publication.

During this period, I spent 1 year in France working with Dr. Bernhard Ryffel (CNRS, Orléans), an experienced pathologist interested in infectious and inflammatory diseases. There, I showed that CCR1, CCR2 and CCR4 receptors play discrete roles in the pathogenesis DENV-2 in our experimental dengue infection model, being involved in the differential activation and accumulation of innate and adaptive immune response leukocytes in target organs. Our major findings can be summarized as follows: **1**) CCR1 does not seem to have

a major role in the pathogenesis of severe experimental dengue infection; **2)** CCR2 appeared to contribute to dengue-associated liver damage and this was reflected on decreased leukocyte activation and decreased lethality. However, there was no major difference in the systemic inflammatory response associated with infection; **3)** CCR4 also contributed to the pathogenesis of experimental dengue infection and was relevant for virus-induced liver damage and associated systemic inflammation. This was reflected on the decreased leukocyte activation and decreased lethality. Overall, these receptors appear not to play an essential role in protection against primary infection, suggesting that the chemokine storm that follows severe primary dengue infection associates mostly to development of disease rather than protection against severe infection (**Publication 13**).

Dr. Ryffel organized an important collaboration with Dr. François Trottein (Institut Pasteur, Lille), to study iNKT cells in dengue infection. Invariant natural killer T (iNKT) cells are a CD1d-restricted T cell population that can respond to lipid antigenic stimulation within minutes by secreting a wide variety of cytokines. This broad functional scope has placed iNKT cells at the frontlines of many kinds of immune responses. Two different aspects provide iNKT cells with a unique ability to influence the immune response. First, their ability to recognize lipids in an antigen-specific manner allows these cells to sample an antigen space that would otherwise be unmonitored by conventional T cells. Second, the kinetics of their responses to antigenic stimulation allow the iNKT subsets to rapidly skew the course of the immune response in directed ways. By establishing an initial path for the immune response, iNKT cells have the potential to dictate how downstream adaptive cells are polarized and, consequently, how they respond (Krovi and Gapin, 2018). Although there are relatively few iNKT cells in the human body (compared to other leukocytes), there is ample evidence for the important antiviral role of these cells (Reilly et al., 2010, Kulkarni et al., 2010). Importantly, the potential role of iNKT cells during flavivirus infection had not yet been addressed, but is well understood for Influenza A infection (Barthelemy et al., 2017, Paget et al., 2012). Coordinating the project with Dr. Joelle Renneson (Institut Pasteur, Lille), we showed a key role for iNKT cells in disease development, morbidity and mortality associated with DENV-2 infection. These cells can accumulate in tissues (liver and spleen), continuously producing pro-inflammatory mediators which ascribe to fatal tissue injury. Moreover, viral clearance is significantly increased in the absence of iNKT cells during infection, suggesting that inflammatory signals are essential for viral proliferation (**Publication 15**). **This conclusion was finally the basis of my PhD thesis.** We are yet to describe whether CC chemokine receptors such as CCR4 and CCR5 might be involved in iNKT trafficking (**unpublished data**).

Having dissected important mechanisms of Dengue disease physiopathology in France, I started to explore new classes of cytokines - Th17 and Th22 - during Dengue infection. IL-22 and IL-17 are cytokines with overall distinct functions. While IL-22 is tissue protective, IL-17 contributes to chemokine production and leukocyte activation in different diseases, from humans to mice (Crome et al., 2010, Afzali et al., 2010, Tian et al., 2013). Furthermore, it has been shown that iNKT cells can abundantly produce IL-17 (Monteiro et al., 2013, Milpied et al., 2011, Doisne et al., 2011, Yoshiga et al., 2008). With an established expertise in the field of Th17 cell function, Dr. Ryffel and I showed that IL-22 is importantly involved in hepatocyte survival upon DENV-2 infection, mainly by maintaining tissue homeostasis, reducing cell death and cytokine production. IL-22 is not involved in viral replication and its signalling is not essential for viral clearance. IL-17 seems to be pro-inflammatory and strongly contributes to lymphocyte and NKT/iNKT activation, and cytotoxic activity in the liver (**Publication 16**).

During this period, 2 grants were acquired with the help of our publications among others from the groups (INSERM/CNPq and INCT/CNPQ). Two MSc students were co-supervised by me during the 4 years of PhD (Rafael Elias and Amanda Coelho), mainly participating in the CCR5 project, a complex and important research with a strong clinical appeal. My period in France, the precious experience in parallel projects and

the close contact with the recent and complex literature of Dengue and flavivirus immunopathology definitely helped me in building a fruitful network of collaborations and a consistent subject for my research career. During 2008, I was selected for **substitute lecturer in Human Physiology and Biophysics** through an open competition (UFMG, Brazil).

Our findings on the immunopathology of *Dengue virus* infection in mice were reviewed in 2014 (**Publication 22**) and summarized in **Figure 6**.

3.9. DSS-induced colitis

Inflammatory bowel diseases (IBD), which include ulcerative colitis (UC) and Crohn's disease, are chronic, relapsing, and remitting inflammatory conditions of unknown origin that affect individuals of both sexes throughout life. Dextran sulfate sodium (DSS) is a sulfated polysaccharide with variable molecular weights. Administration of DSS in drinking water causes human ulcerative colitis-like pathologies in mice due to its toxicity to colonic epithelial cells, which results in compromised mucosal barrier function. DSS colitis, despite its shortcomings, has been of great use in understanding the pathophysiology of intestinal inflammation and, consequently, has informed the theoretical and clinical appreciation of active IBD (Chassaing et al., 2014). This model, in skilled hands, represents a powerful tool with which to study the contribution of any aspect of the increasingly complicated gut environment or to evaluate interventions designed to prevent or ameliorate disease. The involvement of neutrophil granulocytes in the pathogenesis of IBD is well known, but little attention has been given to the role of eosinophils. With the DSS-induced colitis model set up by a colleague in our laboratory, we showed that blockade of eosinophil recruitment with a CCL11-active chemokine-binding protein (Merck-Serono, Switzerland) prevents clinical disease, tissue destruction, and death. (**Publication 6**). Other analyses were carried out in Brazil and Switzerland following the publication. I contributed with the histopathological analysis of the gut and all *in vivo* characterization of the chemokine-binding compound using the pleural and peritoneal cavity in mice.

During my stay in France, I had the opportunity to collaborate in a study involving the role of the Th22 cytokine IL-22 and the antimicrobial peptide Reg3 in experimental colitis, together with Dr. Thomas Secher (INSERM, Tours). We showed that IL-22 is essential for epithelial preservation upon DSS-mucosal injury, and that this cytokine might be involved in intestinal barrier integrity during severe gut inflammation, preventing bacterial translocation and the exacerbation of inflammation (**Secher et al, unpublished data**). Studies published later confirmed our findings.

This experience solidified my expertise in models and diseases where the gut is a major target organ, this time exploring an approach focused in tissue healing and bacterial products in the colon.

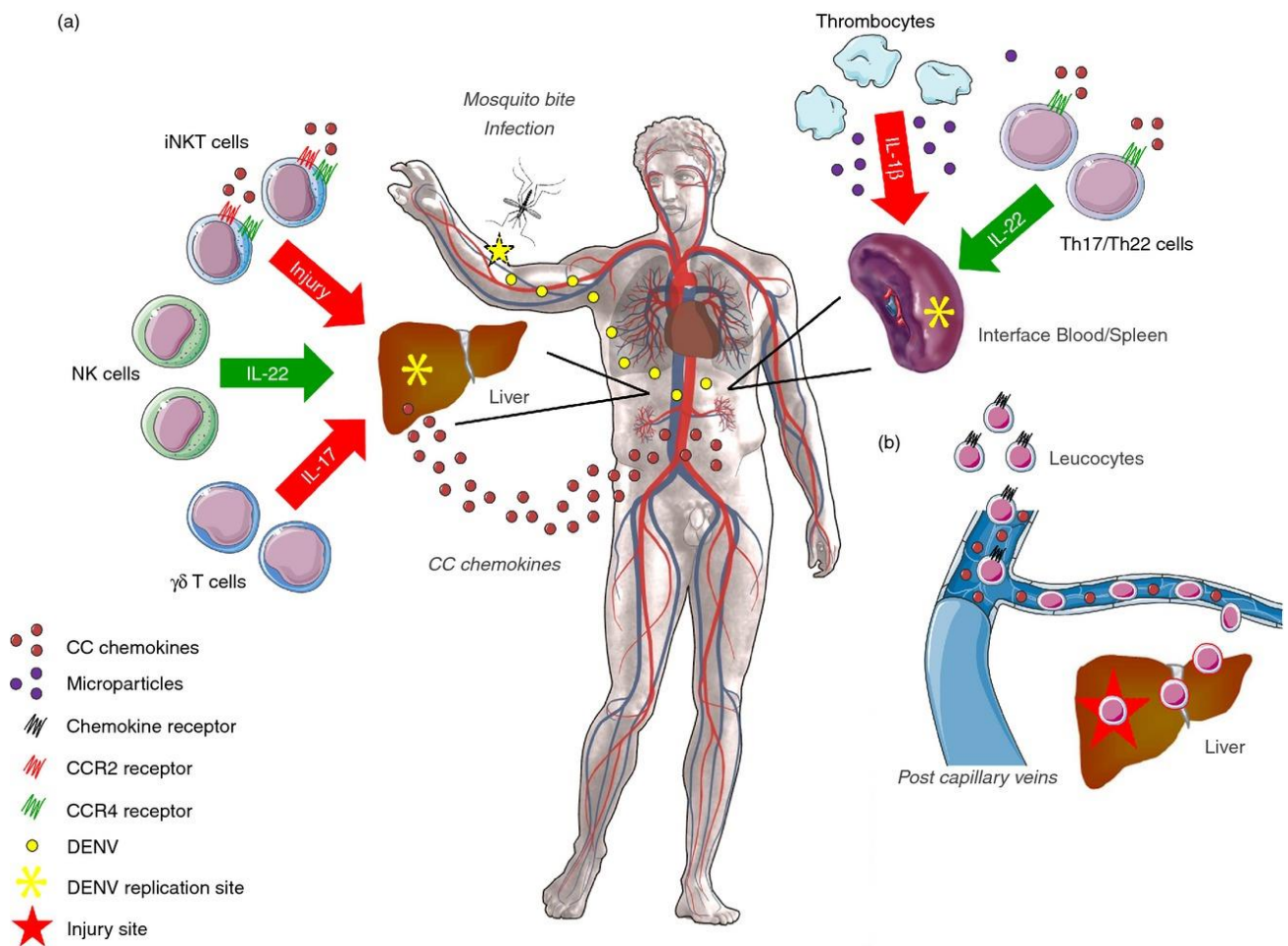


Figure 6. Schematics showing the intricate role of chemokines, cytokines and inflammatory leucocytes in the pathogenesis of dengue virus (DENV) infection. After mosquito bite and infection of host skin and bystander immune cells, certain organs such as liver and spleen will become important sites of viral replication and inflammation. The disseminated inflammatory response observed in dengue haemorrhagic fever/dengue shock syndrome (DHF/DSS) will allow a great production of CC chemokines in blood, liver and spleen. This elevated production of chemokines will contribute to local tissue damage and to the recruitment of different leucocyte populations from the capillary veins into the target organs through the activation of chemokine receptors. The spleen and blood appear to be primary and privileged sites of leucocyte activation, especially for T helper type 17 (Th17)/Th22 lymphocytes and thrombocytes, which can express the CCR4 receptor and produce interleukin-22 (IL-22), or produce IL-1 β and microparticles, respectively. Once leucocytes are activated they come into contact with CC chemokines (b) and are recruited into the liver. (a) Invariant natural killer T (iNKT) cells can be recruited through the CCR2 and CCR4 receptors and play a deleterious role in the liver, greatly contributing to tissue injury. Natural killer (NK) cells are the main sources of IL-22 in the hepatic parenchyma, and appear to play a pro-homeostatic role during DENV infection. $\gamma\delta$ T cells are major sources of IL-17 in the liver, that together with CC chemokines contribute to the massive inflammatory response observed during DHF/DSS. Targeting the CC chemokines, IL-17A and IL-1 family of cytokines may represent an effective adjunct therapy to attenuate the severity of disease manifestations observed in DHF/DSS. (Publication 22). Cover of *Immunology* (Volume141, Issue2, February 2014).

3.10. The role of IL-33 in immunity and inflammation

Prof. Foo Y. Liew, FRS (Glasgow, UK), a recognised immunologist and specialist in allergy and innate immunity (now retired), invited me to develop post-doctoral research in his laboratory (January 2011). We decided to study 2 lines of research: The control of IL-33 functions in Th2 cells by micro-RNAs (miRs) and the role of IL-33/ST2 in mucositis induced by the chemotherapeutic agent Irinotecan (CPT-11). IL-33 is a member of the IL-1 cytokine family, which includes also IL-1 β and IL-18. IL-33 is crucial for the induction of Type 2 immune responses by promoting the synthesis of cytokines such as IL-5 and IL-13 by Th2 lymphocytes, mast cells, basophils and eosinophils (Cayrol and Girard, 2018). IL-33 is also involved in the induction of non-Th2-type acute and chronic inflammation as a pro-inflammatory cytokine. IL-33 signals via a heteromeric receptor that consists of ST2 and IL-1R accessory protein. ST2 (also known as T1), the transmembrane protein encoded by the ST2 gene, is expressed especially on immune cells such as mast cells and activated Th2 cells. The ST2 gene is alternatively spliced to produce a soluble form (sST2), which acts as an IL-33 decoy receptor. IL-33 is produced as a precursor protein (pro-IL-33) that is proteolytically converted to mature IL-33. Both forms are released by necrotic cells and have biological activity. Thus, IL-33 released by necrotic cells during tissue injury may play a DAMP/alarmin-like role in the induction of inflammation (Liew, 2012, Liew et al., 2010). During this time with Prof. Liew, I could expand my knowledge in molecular biology and proteomics by studying miRs in primary Th2 cells. Some potential candidates were identified (**unpublished data**) but I could not explore these data further due to the end of my contract in Glasgow. I was in charge of study design and execution, with the support of the Arthritis and Allergy research groups at the University of Glasgow (Prof. Iain McInnes). The interest now is to check whether some of the identified miRs might be involved in IL-33 signalling during allergy, an inflammatory phenomenon mostly driven by Th2 cells.

Based on my previous knowledge on inflammatory responses taking place in the gut, Dr. Liew and myself were interested in investigating the involvement of the alarmin/cytokine IL-33 and its receptor ST2 in mucosal damage induced by Irinotecan (CPT-11). Irinotecan inhibits the action of topoisomerase I and prevents religation of the DNA strand by binding to topoisomerase I-DNA complex. The formation of this ternary complex interferes with the moving replication fork, which induces replication arrest and lethal double-stranded breaks in DNA. Mucositis is a common and serious problem in patients undergoing cancer chemotherapy, ascribing to diarrhoea, mucosal damage, neutropenic fever, body weight loss and eventually sepsis (Boeing et al., 2021, Gibson et al., 2007). Mucositis develops as a consequence of epithelial injury. However, its pathophysiology is complex and involves multiple steps including the generation of reactive oxygen species (ROS) and reactive nitrogen species, together leading to epithelial damages. Chemotherapy directly causes DNA damage and cell death with activation of NF κ B and up-regulation of cytokine production (**Figure 7**). In the ulcerative phase, epithelial erosion can lead to risk of microbial infiltration and septic shock. Tissue damage in early stages of chemotherapy-induced mucositis resembles a lot to that found for bleomycin-induced lung injury or i-I/R.

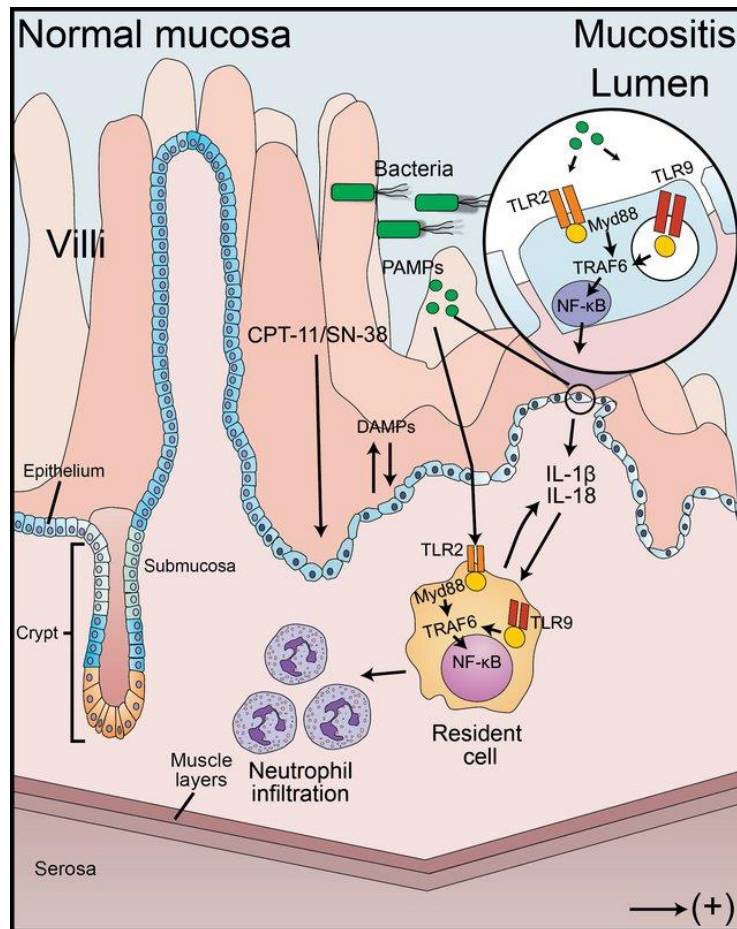


Figure 7. Hypothesis for the development of intestinal mucositis. Irinotecan (CPT-11) is metabolized in the liver into the active compound SN-38, which in turn is inactivated through glucuronidation to SN-38G. SN-38G is eliminated through the common bile duct into the intestinal tract where it is re-activated by Gram-negative bacteria containing beta-glucuronidase enzymes. In the intestinal lumen, the active SN-38 leads to epithelial damage, allowing the enteric bacteria to translocate. Pathogen-associated molecular patterns (PAMPs) and Damage-associated molecular patterns (DAMPs) are recognized by the toll-like receptors, which signal through the MyD88 adaptor protein and TNF receptor-associated factor 6 (TRAF6) to activate NF-κB and cytokine synthesis. This process contributes to neutrophil recruitment to the site of infection, amplifying the damage (Wong et al., 2015).

We initially showed that ST2 blockade prevents mucositis in mice, and this is mostly regulated by a reduced neutrophil influx through the CXCR2 chemokine receptor to the damaged mucosa, and to the upregulated production of IL-10 in the absence of ST2 (**Publication 21**). IL-33-treated mice developed more severe disease, with strong accumulation of CXCR2⁺ neutrophils, CD86⁺ M1 macrophages, apoptosis, bacterial translocation and reduced tissue repair. This crosstalk between IL-10, chemokines and CXCR2⁺ neutrophils was already known in my previous projects involving similar experimental models (i-I/R, colitis, pulmonary fibrosis). We also showed that blocking ST2 during cancer chemotherapy relieves the aforementioned side effects while allowing effective tumour reduction (ectopic colon carcinoma CT26) (**Publication 21**).

In collaboration with Dr. Damo Xu (University of Glasgow, UK), we showed in the bleomycin model of lung fibrosis in mice that IL-33 released by alveolar macrophages and lung epithelial cells drives lung inflammation and fibrosis through early recruitment of type 2 innate lymphoid cells (ILC2) and neutrophil accumulation into the lung parenchyma (**Publication 23**). I had first authorship (one as corresponding author) in both works. In parallel, I helped on investigating the role of IL-33 in experimental cerebral malaria (ECM),

where we provided evidence that IL-33 can prevent the development of ECM by orchestrating a protective immune response via ILC2, M2 macrophages and regulatory T cells (Tregs) (**Publication 26**). I was second author in this publication.

With experience on the IL33/ST2 axis in infection, immunity and inflammation, I started a semi-independent project on the role of IL-33 and ST2 in the immunopathology of DENV infection, with the full support of Dr. Liew and Dr. Ryffel. We demonstrated that IL-33 plays a disease-exacerbating role during experimental dengue infection in immunocompetent mice (**Publication 38**). Mice infected with DENV-2 produced high levels of IL-33. DENV2-infected mice treated with recombinant IL-33 developed markedly more severe disease compared with untreated mice as assessed by mortality, granulocytosis, liver damage and pro-inflammatory cytokine production. Conversely, ST2^{-/-} mice (deficient in IL-33 receptor) infected with DENV2 developed significantly less severe disease compared with wild-type mice. Furthermore, the increased disease severity and the accompanying pathology induced by IL-33 during dengue infection were reversed by the simultaneous treatment with a CXCR2 receptor antagonist (DF2156A). Together, these results indicated that IL-33 plays a disease-exacerbating role in experimental dengue infection, probably driven by CXCR2-expressing cells, leading to elevated pro-inflammatory response-mediated pathology. This project kept my association with Orléans and Glasgow quite tight, even after the end of my contract and first years at the INRAE.

3.11. Initial developments at the INRAE and avian colibacillosis

By mid-2013 I was recruited as a *Chargé de Recherche* (CRCN) at the INRAE, formally starting my work at the UMR ISP in Nouzilly in January 2014. The Research Unit UMR ISP (Infectiologie et Santé Publique) at Nouzilly dedicates its research on infectious diseases representing threats for animal and/or human health (the One Health concept). There were a growing interest and full support of the direction (Dr. Nathalie Winter) for the development of more mechanistic studies on avian immunology and on colibacillosis. My recruitment was directly related to this increasing interest. The UMR ISP is composed by excellent teams studying different aspects of host response to avian diseases, a reference in France. Team PCA (Pathogénie de la Colibacillose Aviaire), led by Dr. Catherine Schouler, had expertise in molecular epidemiology and characterization of Avian Pathogenic *Escherichia coli* (APEC) strains including analysis of bacterial adhesion and invasion to host tissues and cells. I was recruited to team PCA as an immunopathologist, with expertise in *in vivo* and *in vitro* models for the study of infection and inflammation. I found the topic extremely interesting, especially on the possibility of expanding my horizons in immunological mechanisms operating in non-mammalian species. A nice opportunity to develop a new topic in a mostly neglected area of research, which could mean less competition in the field and a completely new network of colleagues.

Avian pathogenic *Escherichia coli* (APEC) is the etiological agent of colibacillosis. APEC infection leads to increased mortality and decreased egg production in poultry. Therapy is mainly based on antibiotherapy and current vaccines have poor efficacy. To date, the role of immune cells and mediators of inflammation in colibacillosis is limited to very descriptive studies (**Publication 28**) (**Figure 8**).

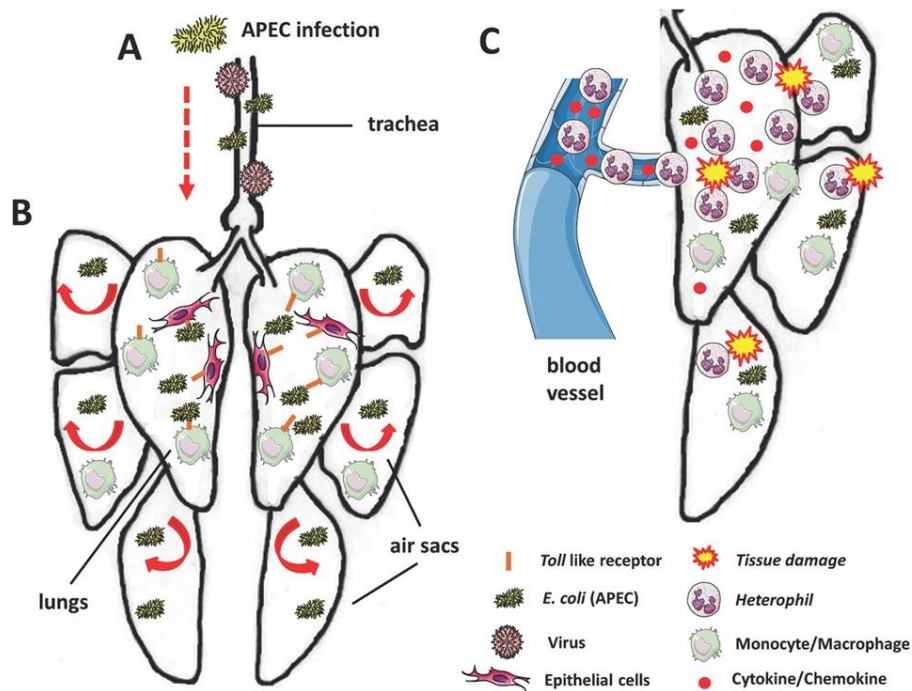


Figure 8. Schematics showing chicken's inflammatory response to APEC in the respiratory tract. (A) Upon inhalation of contaminated aerosol particles, APEC might interact with and infect epithelial cells already in the trachea. A previous viral infection, such as IBV, may damage the respiratory mucosa and facilitate APEC colonization. (B) Resident cells such as epithelial cells and macrophages in lungs and air sacs will be the first line of defense through recognition of bacterial PAMPs by TLRs or bacteria phagocytosis. (C) These responses will result in proinflammatory cytokines and chemokines production that will activate and/or recruit other leukocytes to the infected site. Inflammation will also contribute to tissue damage and impairment of lung function if bacterial colonization persists. Heterophils are likely to be the first leukocyte population to be recruited from the bloodstream to the infected site by chemokines within few hours after the establishment of infection. Later in the inflammatory response to APEC, monocytes arrive in the second wave of leukocyte immigration into the lungs not only to optimize bacterial clearance but also to contribute to phagocytosis of dying heterophils and thus contribute to tissue repair. The kinetics of these cellular events and the mediators of inflammation that participate in the immigration and activation of heterophils or monocytes/macrophages in APEC-infected chicken lungs remain largely unexplored (**Publication 28**).

The route of infection seems to be of respiratory and vaginal origin. Omphalitis and yolk sac infection occurs by fecal contamination of eggs or *in ovo* during egg formation when laying hens suffered SPS (Antao et al., 2008). Furthermore, as a secondary opportunistic bacterium, *E. coli* can play a role in some bone and joint infections affecting poultry flocks. APEC is assumed to cross the respiratory epithelia and penetrate deeply into the mucosa and submucosa to reach blood stream causing septicemia. Birds surviving to septicemia develop subacute fibrinopurulent airsacculitis, pericarditis and perihepatitis. Although airsacculitis is observed, it is unclear whether it results from primary respiratory exposure or from extension of polyserositis. Furthermore, sequelae of colisepticemia could also lead to arthritis, osteomyelitis, peritonitis and salpingitis.

Bird's lungs possess parabronchi, a structure that allows airflow to pass through the lung in one direction. Parabronchi are in close contact with blood capillaries, an important area for gas exchange. In the avian respiratory system, air moves in and out through distention and compression of the air sacs, not the lungs, as observed in mammals (**Figure 9**). The air sacs fill a large area of the chest and abdominal cavity, being located at the ends of the airway system. At any given moment, air may be flowing into and out of the lung, but also staying in the air sacs during the whole process. These peculiar anatomical features may strongly favor

bacterial colonization of bird's lower airways (Reese et al., 2006), one of the reasons why the pulmonary form of colibacillosis is highly prevalent. The gas-exchange regions of the lung and the air sacs are the main sites of bacteria entry into the bloodstream. However, resident cells might help recognition and elimination of invading bacteria. In avian lungs, both macrophages and dendritic cells are present in the mucosa of larger airways (de Geus and Vervelde, 2013). These phagocytic cells are located at these strategic check points where fresh air is distributed into the gas exchange areas. Air sacs are also relatively vulnerable to colonization and invasion due to an apparent lack of robust resident phagocytic cells. Even if air sacs do possess resident cells, their phenotypic characterization, numbers and relevant differences to those phagocytic cells present in lung parenchyma remain to be further studied (Dziva and Stevens, 2008, Reese et al., 2006).

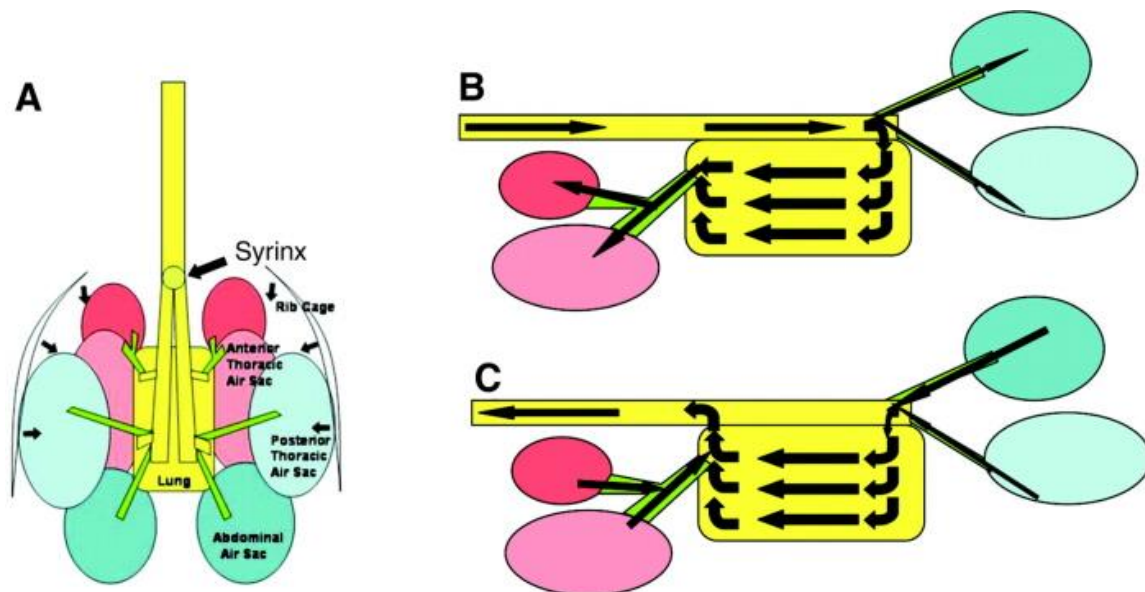


Figure 9: A schematic of the avian respiratory system, illustrating the major air sacs and their connections to the lung. (A) The lateral and dorsal direction of motion of the rib cage during exhalation is indicated by arrows. (B) The direction of airflow during inspiration. (C) The direction of flow during expiration. Respiration in birds requires two respiratory cycles (inspiration, expiration, inspiration, expiration) to move the air through the entire respiratory system. In mammals, only one respiratory cycle is necessary (Plummer and Goller, 2008).

My first year at the INRAE was dedicated to get familiar to tools, reagents and particularities of avian immunology in order to start more precise work on colibacillosis. With the help of a Master 2 student (Kathleen Proust), we set up or improved different cell culture conditions (chicken cell lines and primary cells), molecular biology techniques, biochemical tests, qPCR primer probes targeting different elements of the chicken immune system, and other routine techniques that are now being fully utilized in ours and other teams at the UMR ISP. In addition, I participated in setting up different experiments and I've got used to *in vivo* protocols of APEC infection (subcutaneous, intra-tracheal or intra air-sac infection) in chickens, including a lethality test in 1-day old chicks, which is useful to study drugs and reagents with a therapeutic potential.

The collaboration with important avian immunologists such as Prof. Thomas Göbel (LMU, Germany), Prof. Bernd Kaspers (LMU, Germany) and Dr. Lonneke Vervelde (The Roslin Institute, UK) was essential for the acquisition of tools and for the exchange of experimental protocols in this beginning. Since then, these collaborations remain highly active. During this period, we wrote a "kick-off" review where we discussed current knowledge on APEC diversity and virulence, including host response to infection and the associated inflammatory response, with a focus on pulmonary colibacillosis (**Publication 28**).

This period of development and adaptation was essential to better understand the team's needs and find a research niche to start developing new studies on the chicken immune response. Team PCA was rather new upon my arrival (it was created in 2012) and many essential and more complex questions regarding host's immune response to colibacillosis were unresolved and are still being developed, which requires time. I therefore decided to use my past scientific background to start developing short to mid-term studies that would bring novelty to the field, solidify techniques and expertise within the UMR ISP, open doors to potential therapeutic targets, and provide solid data for quick publications that would in a mid-term favor my participation in national grant applications and big European consortiums.

I initially aimed to investigate vascular mediators involved in the early host response to infection, which are closely associated to tissue injury and bacterial clearance, notably Platelet Activating Factor (PAF) and Ornithokinin (OK, the avian analog of mammalian Bradykinin). These vascular mediators are quickly produced (within an hour) at infected sites and play a role in the early development of local inflammatory responses. The study on molecules participating on the interface blood-tissue during infection in birds remains largely undeveloped and is an excellent research field in the context of avian immunology. Having already worked with lipid mediators, angiogenesis and cell-tissue interactions, my interest on the role of PAF and OK in the context of colibacillosis was justified. Since team PCA is interested in early events of bacteria adhesion and colonization of the chicken respiratory tract, the study on vascular mediators with immune modulating effects could bring important insights not only to the comprehension of colibacillosis pathogenesis in poultry but to the host-pathogen interaction in chickens.

3.12. Implication of the platelet activating factor (PAF) system in the host immune response to *Escherichia coli* infection in chickens

The phospholipid Platelet Activating Factor (PAF) is one of the most studied lipid mediators in the context of tissue- and vascular-related inflammatory responses in mammals (Zimmerman et al., 2002, Camussi et al., 1990, Spina et al., 1989). In bacterial and viral infections, PAF production and signaling trigger exacerbated innate immune responses, thus leading to inflammation and collateral tissue damage (Souza et al., 2009, Caini et al., 2007, Villani et al., 1991). Additional functions for PAF/PAFR signaling suggest a pivotal role in macrophage resistance to bacterial infection. A drug targeting PAFR was used in the past to treat asthma (Modipafant[®], Pfizer).

The PAF system (receptor and enzymes involved in its metabolism and catabolism) is phylogenetically conserved and genes encoding components of the PAF system are present in the chicken genome. One study in 1985 showed that chicken thrombocyte activation by PAF required Ca²⁺ and appeared to be mediated through a specific receptor for PAF (Cox, 1985). However, no other study has so far addressed the role of the PAF system in other cell types or disease contexts in birds.

Our study revealed that chicken macrophages express PAFR at transcript and protein levels. Also, *E. coli* LPS was able to induce upregulation of PAFR expression. Exogenous PAF induced release of reactive oxygen species (ROS) and promoted phagocytosis of zymosan-beads by chicken macrophages. PAF also synergized with LPS to upregulate COX-2 expression, as previously observed in mammalian cells, together with nitric oxide (NO) production and pro-inflammatory cytokine/chemokines expression upregulation. These effects were reverted by PAFR blockade with PCA 4248 (a PAFR antagonist) and were Calcium Calmodulin (CamKII)-dependent. PAF also favored bacterial killing in infected chicken macrophages in an *in vitro* infection model using a highly resistant/adherent APEC strain (MT78). In chicken primary endothelial cells, PAF directly increased cell permeability and synergized with LPS or APEC to promote upregulation of cytokines/chemokines expression (**Publication 35**). These latter interesting data was obtained in collaboration with Dr. Sascha Trapp

and Dr. Pascale Quéré (at that time part of team PIA, UMR ISP), a collaboration that would soon evolve into a very close partnership.

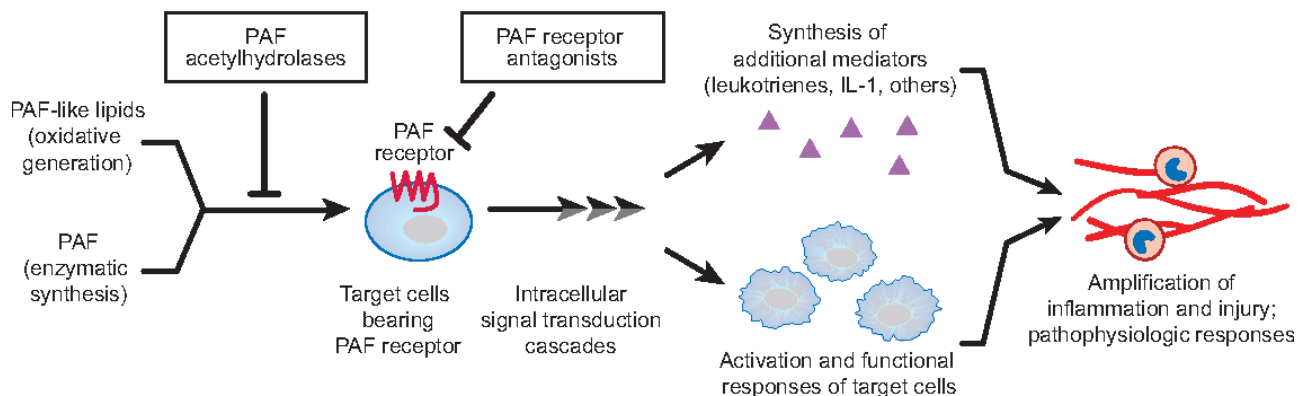


Figure 10. The PAF signaling cascade. The PAF signaling system includes PAF and PAF-like lipids, which are phospholipid ligands, and a G-protein-coupled receptor, the PAFR, that has restricted distribution on target inflammatory, immune, and hemostatic cells. Engagement of the receptor triggers cellular activation and, via intracellular signaling cascades, alterations in cellular phenotype and function. A variety of regulatory mechanisms have evolved to control the PAF signaling system, including PAFR downregulation and desensitization, intracellular biochemical modulation, and activities of a family of enzymes e the PAF acetylhydrolases that selectively degrade PAF and PAF-LL. Plasma PAF AH, a secreted form, is constitutively present in blood under basal conditions and limits the half life of circulating PAF to minutes. Recombinant PAF AH and competitive PAFR antagonists have been studied as candidate therapies for sepsis in experimental models and clinical trials (Montrucchio et al., 2000).

In the lungs of APEC infected chickens, PAFR is greatly upregulated, together with Lyso-PAFAT, a key enzyme of its biosynthesis, suggesting that PAF synthesis and the likelihood of PAF to signal through its receptor is increased during colibacillosis. In a lethality test using 1-day old chicks infected with an APEC strain, animals receiving PCA 4248 (a PAFR antagonist) orally at 10 mg/kg after infection displayed 20% average reduced lethality as compared to infected-untreated controls (**unpublished data**).

In summary, PAF promotes and/or potentiates the inflammatory response induced by bacteria or LPS in chicken macrophages. *In vivo*, PAF appears to be linked to exacerbation of inflammation during bacterial infection. These data reinforces the relevance of studying the PAF system, and maybe other lipids, in the context of chicken colibacillosis and other infectious diseases of poultry. The use of PAFR antagonists *in vivo* to limit colibacillosis-derived collateral inflammation still need to be validated but might suggest a therapeutic strategy. This project received financial support from the Departement Santé Animale (INRAE).

3.13. Unveiling the participation of avian kinin ornithokinin (OK) and its receptors in the chicken inflammatory response

In line with the role of PAF in modulating early events linked to the upregulation of chicken's innate immune response during infection and inflammation, I decided to investigate the kinin/kalikrein (K/KLK) system in the avian context. The K/KLK system consists of a phylogenetically ancient cascade that includes substrates (kininogens), proteases (kallikreins), biologically active peptides (kinins) and kinin receptors (**Figure 11**). Bradykinin (BK) is the main vasoactive peptide of the kinin group of proteins. It acts through B1R and B2R

receptors in mammals and is regarded as an important mediator of edema, shock, and inflammation during sepsis (Ding et al., 2019, Fischer et al., 2004, Fink, 1998). In mammals, BK is known to activate members of the mononuclear phagocyte system such as dendritic cells (DCs), monocytes and macrophages for the production of pro-inflammatory mediators (Ricciardolo et al., 2018, Landgraf et al., 2004, Eric et al., 2003, Stewart et al., 2002). Mammalian B1R and B2R are expressed in mononuclear phagocytes depending on species, tissue, and differentiation/activation stage. BK stimulates TNF- α and IL-1 β release from murine macrophages (Bockmann et al., 1998, Bockmann et al., 1997, Sato et al., 1996, Bockmann and Paegelow, 1995). Drugs targeting this system are already available in a clinical context (i.e. Firazyr/Icatibant[®], a B2R antagonist).

The K-KLK system has received relatively little attention in non-mammalian vertebrates. Kinin peptides and their receptors are conserved in most vertebrates, although their physiological and pathological functions are mostly unknown outside humans and rodents (Ponczek et al., 2020, Conlon, 1998). In birds, incubation of chicken plasma with pig pancreatic kallikrein and/or trypsin generated a peptide with BK-like activities (Hochstrasser and Werle, 1966). Later, Kimura and colleagues showed that purified chicken kininogen is rapidly degraded by bovine plasma kallikrein and release ornithokinin (OK), whose primary structure was determined as Arg-Pro-Pro-Gly-Phe-Thr-Pro-Leu-Arg, similar to BK except for the substitution of Thr-6 and Leu-8 for Ser-6 and Phe-8 (Kimura et al., 1989). A proenzyme similar to human plasma prekallikrein was also isolated from chicken plasma (Schleuning et al., 1983). OK, but not BK, induces contraction of chicken smooth muscle and has strong hypotensive effect in chickens (Prezoto et al., 2009). However, no study has evaluated so far the participation of chicken K-KLK system components in the inflammatory response. This system remains largely unknown in birds.

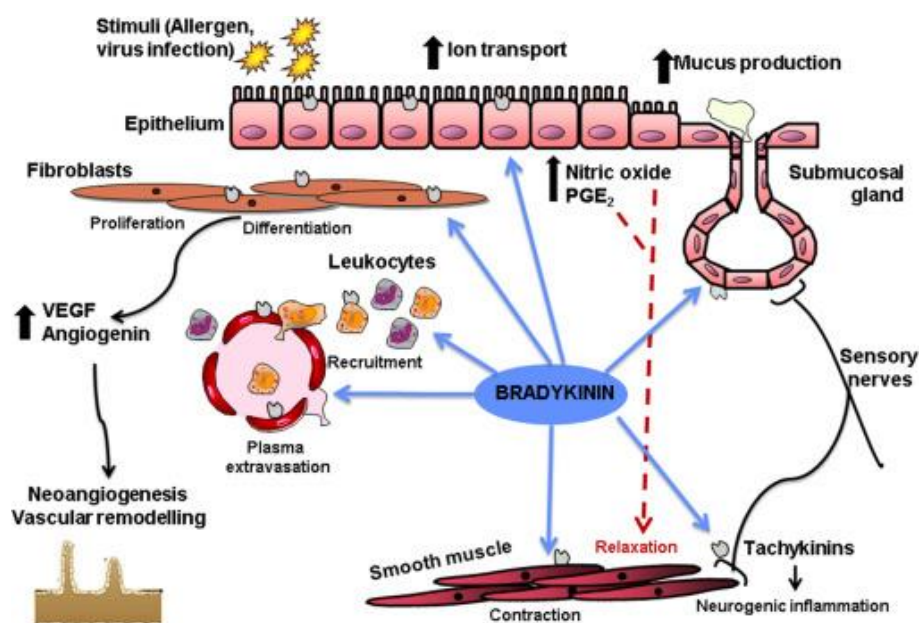


Figure 11. In airway epithelial cells, bradykinin stimulates the release of bronchorelaxing factors PGE₂ and nitric oxide, the mucus secretion from human submucosal glands and the ion transport. Bradykinin is a potent inducer of airway microvascular leakage and vascular permeability and causes prolonged leakage at all airway levels. Moreover, it has direct effects on the recruitment and activation of inflammatory cells. Upon stimulation of bradykinin receptors, fibroblasts activation provokes the release of vascular growth factors (VEGF and angiogenin) able to modulate bronchial vascular remodelling in asthma. In airway smooth muscle bradykinin induces bronchoconstriction via direct stimulation of its receptors and via indirect neural activation (cholinergic nerves and C-fibre sensory nerves); nevertheless, epithelium-derived PGE₂ and nitric oxide reduce excessive bronchoconstriction in asthma (Ricciardolo et al., 2018).

We therefore showed that B1R, B2R and kininogen 1 (KNG1) are expressed in unstimulated chicken tissues and macrophages. We next showed that chicken B1R and B2R are expressed at transcript and protein levels in chicken macrophages and are upregulated by *E. coli* LPS or avian pathogenic *E. coli* (APEC) infection. Interestingly, exogenous OK treatment induced internalization and degradation of OK receptors protein, notably B2R. Also, OK induced intracellular calcium increase and potentiated zymosan-induced ROS production and Dextran-FITC endocytosis by chicken macrophages. Exogenous OK itself did not promote APEC killing and had no pro-inflammatory effect. However, when combined with LPS or APEC, OK upregulated cytokine/chemokine gene expression and NO production by chicken macrophages. This effect was not blocked by canonical non-peptide B1R or B2R receptor antagonists but was GPCR- and PI3K/Akt-dependent. *In vivo*, pulmonary colibacillosis led to upregulation of OK receptors expression in chicken lungs and liver. Also, colibacillosis led to significant upregulation of the OK precursor KNG1 expression in liver and in cultured chicken hepatocytes (LMH) (**Publication 32**).

We therefore provided hitherto unknown information on how OK and its receptors can modulate (or are modulated) during inflammation and infection in chickens, thus highlighting the interest in studying the K-KLK system and other neglected early mediators of inflammation in birds (. Part of this project was developed together with a Master 2 student (Mélanie Pinaud). We would appreciate to gain information on the production of OK *in vivo* in chickens and this study might be performed in a near future. Also, it would be interesting to perform *in silico*, docking analysis, to identify B1R or B2R receptor antagonists that would better work in OK receptors. The same applies to the PAF receptor (PAFR).

I believe that the work performed with PAF and OK allowed us to identify and associate, for the first time, key components of the cardiovascular system to the host immune response to bacteria in an economically relevant animal model. These works resulted in two publications, included the development of *in vitro* approaches on avian immunology, have potential long-term therapeutic interest, and brought new information to the avian immunology and poultry veterinary community. In addition, it helped me solidify new expertise on chicken immunology acquired upon my arrival at INRAE and pave the basis to a promising start as a researcher at the UMR ISP. This experience revealed critical to the management and optimization of new projects and collaborations that took place during these studies, which will now be presented below.

3.14. Impact of avian influenza virus infection on the chicken mucosal immune response to respiratory colibacillosis (MICHIC project)

Together with Dr. Sascha Trapp (at this time in team PIA), Dr. Schouler established a collaboration with The Roslin Institute, UK and the LMU, Germany, for the submission of a grant proposal (ANIHWA/ERA-NET) in 2014. I could therefore participate in the elaboration of the work packages in order to add expertise and to open new research possibilities. The project MICHIC: Understanding mucosal immunology and co-infections in the chicken to drive vaccine strategies received funding for 3 years (2015-2018).

Colibacillosis in poultry is frequently associated with respiratory viral infections, such as Infectious Bronchitis Virus (IBV) infection. Low pathogenic avian influenza viruses (LPAI) are highly infectious respiratory pathogens that usually cause mild clinical disease in poultry. Their occurrence in poultry farms is largely underestimated since farmers are not obliged to declare the disease. The physiopathology of co-infections with respiratory viruses (notably LPAI) and bacterial pathogens in chickens remains poorly understood. Furthermore, early innate immune responses to bacteria have previously been shown to be compromised by the preceding onset of a Type I interferon (IFN) response induced by influenza viruses in humans (**Figure 12**) (Rynda-Apple et al., 2015, Boxx and Cheng, 2016, Parker, 2017, Barman et al., 2021). It is speculated that type

I IFNs may modulate macrophage and granulocyte responses that otherwise assist in the clearance of bacteria from the lungs (Connolly and Hussell, 2020).

In this project, we exploited techniques and tools that were previously established at teams PCA and PIA during multiple APEC/LPAI mono-infection studies. By employing a number of different assays (e.g. bacteriology, qRT-PCR, histopathology, flow cytometry/FACS) we assessed the relationships between the outcome of APEC super-infection and LPAI-induced alterations of the chicken respiratory immune homeostasis. Furthermore, we established and exploited an *ex vivo* co-infection model based on precision-cut lung slices (PCLS) from inbred chickens.

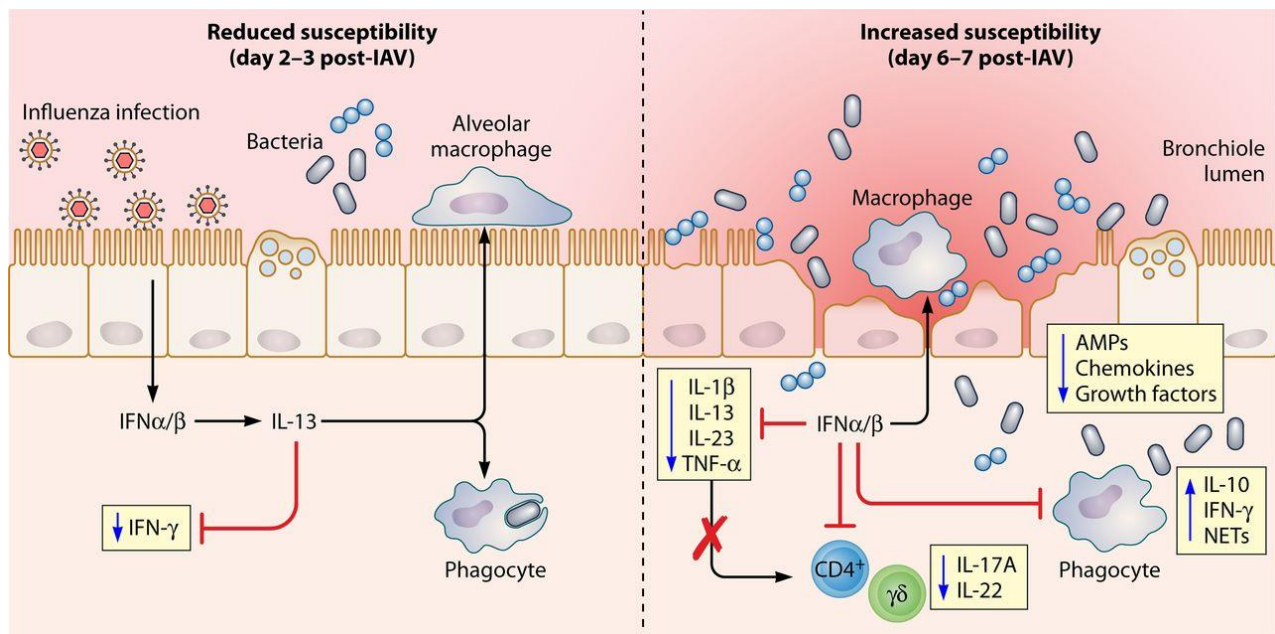


Figure 12. Common pathways of susceptibility to postinfluenza bacterial superinfections in mammals. Early after influenza virus infection, mice show reduced susceptibility to superinfection that is at least in part due to increased production of IL-13. This IL-13-rich environment does not permit IFN- γ production, allowing unaltered phagocytosis and clearance of bacteria. The role for either neutrophils or macrophages (phagocytes) in bacterial clearance early during influenza virus infection has not been fully investigated. Progression of influenza virus infection results in increased susceptibility to secondary infection. Type I IFN (or IL-27) signaling initiated in response to influenza virus infection results in downregulated production of IL-1 β and IL-23 and impaired type 17 immune responses. Inhibition of IL-17 and IL-22 reduces production of antimicrobial peptides. Type I IFN signaling also reduces levels of neutrophil chemoattractants Cxcl1 and Cxcl2 and can induce formation of NETs. IL-27 induced during influenza virus infection further suppresses IL-17 production but stimulates production of regulatory cytokine IL-10, which contributes to increased susceptibility to superinfection, presumably by alteration of the anti-influenza inflammatory response. IAV, influenza A virus infection (Rynda-Apple et al., 2015).

The avian respiratory tract is a common entry route for many pathogens and an important delivery route for vaccination in the poultry industry. Immune responses in the avian lung have mostly been studied *in vivo* due to the lack of robust, relevant *in vitro* and *ex vivo* models mimicking the microenvironment. PCLS have the major advantages of maintaining the 3-dimensional architecture of the lung and includes heterogeneous cell populations. PCLS have been obtained from a number of mammalian species and from chicken embryos. However, as the embryonic lung is physiologically undifferentiated and immunologically immature, it is less suitable to examine complex host-pathogen interactions including antimicrobial responses. In MICHIC, we set up a protocol to prepare PCLS from immunologically mature chicken lungs

(Publication 42). With the protocol set up, we tested different culture conditions, and found that serum supplementation has a detrimental effect on the quality of PCLS. Viable cells in PCLS remained present for ≥ 40 days, as determined by viability assays and sustained motility of fluorescent mononuclear phagocytic cells. The PCLS were responsive to lipopolysaccharide stimulation, which induced the release of nitric oxide, IL-1 β , type I interferons and IL-10. Mononuclear phagocytes within the tissue maintained phagocytic activity, with live cell imaging capturing interactions with latex beads and an APEC strain (MT78). Finally, the PCLS were also shown to be permissive to infection with low pathogenic avian influenza viruses. We therefore established a model of immunologically mature chicken PCLS to simulate live organ responsiveness and cell dynamics, which can be readily exploited to examine host–pathogen interactions and inflammatory responses that take place in the chicken lung (**Publication 42**).

A PhD student, Damien Garrido, was recruited to work in MICHIC. I supervised Damien during his thesis (Dr. Schouler, being HDR, was the thesis director), where we explored the effects of type I IFNs on the outcome of bacterial challenge in chicken macrophages. We discovered that chicken IFN α priming boosted avian pathogenic *E. coli* infection- or LPS-induced ROS/NO production and led to an increased transcriptional expression (and protein production) of *NOS2*, *IL1B*, and especially *IFNB*. Strikingly, neutralization of IFN β during bacterial/LPS challenges limited IFN α -induced augmentation of the bacterial-driven pro-inflammatory response. Bacterial uptake was also increased in IFN α -primed macrophages. Moreover, IFN α and IFN β induced differential gene expression profiles and activated different intracellular signaling pathways. We therefore evidenced that an IFN α -enriched environment exacerbates the pro-inflammatory response of chicken macrophages to bacterial infection via IFN β upregulation and signaling, thus shedding light on immunological mechanisms underlying viral/bacterial superinfections in birds (**Publication 37**)

It was a great opportunity to exchange knowledge with the European collaborators and to improve social and management skills with Damien Garrido, PhD student. We have published 2 articles within the MICHIC project and Damien collaborated in 2 other articles from my individual projects (PAF and OK). Another manuscript is still under preparation (GM-CSF and macrophage differentiation in blood).

3.15. DNA sensing and innate immunity in chickens

By the end of 2015, Dr. Brian Ferguson (Department of Pathology, University of Cambridge, UK) contacted me for a collaboration. Dr. Ferguson is a specialist in DNA “sensing” in mammals. He is particularly interested in how the detection of nucleic acids triggers an innate immune response in different species, such as the chicken.

The pattern recognition receptors (PRRs) that directly sense the presence of foreign DNA inside host cells are critical for mounting a rapid and effective innate immune response (Jeffries and Marriott, 2020, Bhat and Fitzgerald, 2014, Keating et al., 2011). The anti-viral immune response is dependent on the ability of infected cells to sense foreign nucleic acids. In multiple species, the pattern recognition receptor (PRR) cyclic GMP-AMP synthase (cGAS) senses viral DNA as an essential component of the innate response. cGAS initiates a range of signaling outputs that are dependent on generation of the second messenger cGAMP that binds to the adaptor protein stimulator of interferon genes (STING) (Barber, 2014, Diner and Vance, 2014, Panne, 2013, Civil et al., 2013).

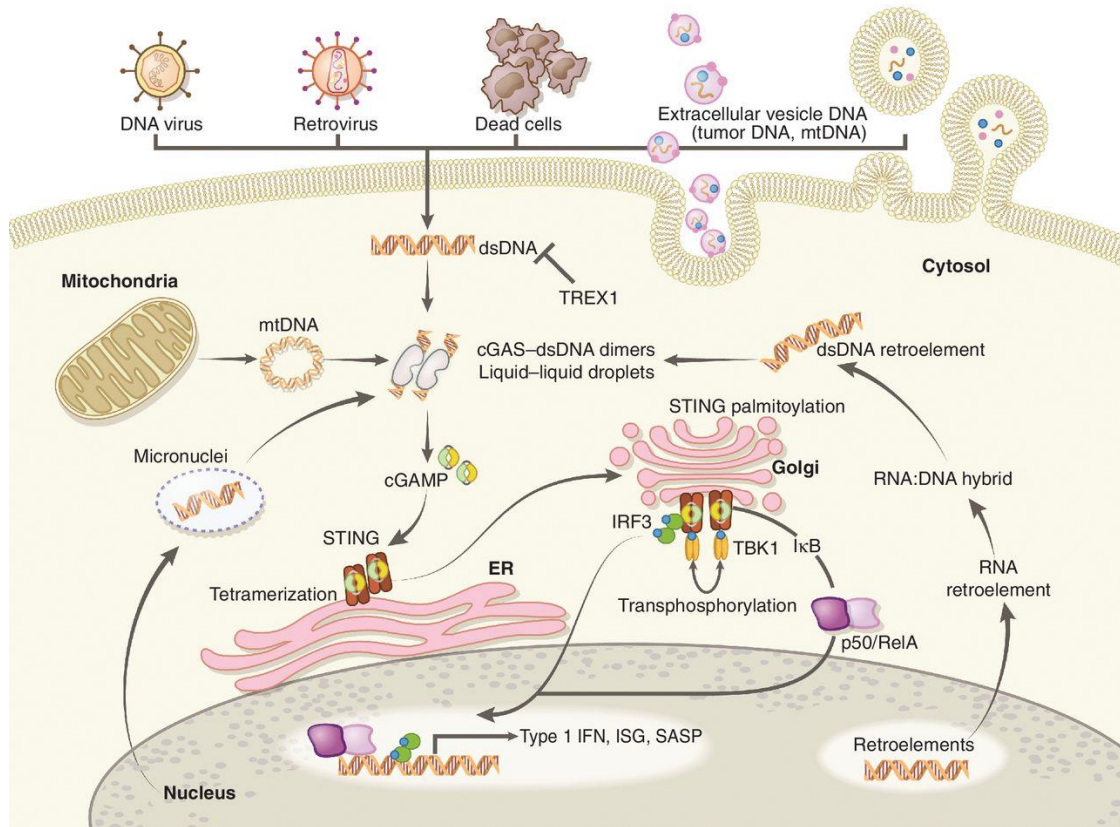


Figure 13. cGAS–STING signaling in immunity. cGAS is an innate immune sensor that recognizes a diverse array of cytosolic dsDNA, which includes DNA with viral, apoptotic, exosomal, mitochondrial, micronuclei, and retroelement origins. cGAS oligomerizes with dsDNA in a 2:2 complex. The interaction of cGAS with DNA induces the formation of liquid droplets through phase transition, in which cGAS exerts its catalytic role to generate the second messenger 2',3'-cGAMP. The presence of cGAMP stimulates STING at the ER, which undergoes higher-order tetramerization. STING translocates from the ER to Golgi compartments and is palmitoylated. STING serves as a signaling platform for TBK1 and IKK. TBK1 phosphorylates STING, which in turn recruits IRF3 for TBK1-mediated phosphorylation. Activated IRF3 dimerizes and translocates to the nucleus. IKK-mediated phosphorylation of the inhibitory IκB protein licenses nuclear entry of p50-RelA. Together with IRF3 in the nucleus, p50-RelA dimers stimulate the transcriptional expression of IFN and other immune-stimulatory genes. (Kwon and Bakhom, 2020)

Despite being extensively studied in mammalian systems, the PRRs that sense pathogen's DNA are not well defined in chickens and their function in the defense against pathogens poorly characterized. In this collaboration, we aim to identify the PRRs responsible for sensing intracellular DNA and analyze the innate sensing pathways that they trigger. There are two main key signaling outputs that result from DNA-PRR stimulation: 1) The activation of interferon regulatory factor (IRF)-dependent Type I IFN and chemokine transcription and 2) Inflammasome-dependent interleukin-1beta (IL-1β) processing and secretion.

We already evidenced that key molecules of the DNA sensing pathway such as cGAS, STING, TBK1 and IRF7 are expressed in chicken epithelial cells, fibroblasts and macrophages. Interestingly, STING, TBK1 and IRF7 are upregulated if cells are treated with Type I IFN (IFNα), simulating a pro-inflammatory milieu. These cells, and more notably macrophages, are also able to respond to transfected DNA by producing cytokines and chemokines. This phenomenon is inhibited if we block the kinase TBK1 with a specific inhibitor, suggesting this pathway is conserved in chickens. Also, activation of STING with cGAMP (a cGAS product) promotes a pro-inflammatory response through cytokines/chemokines production. Funding from the BBSRC (UK) was granted in July 2018, with Dr. Brian Ferguson as the principal investigator (PI). I am work-package leader for the studies

using primary chicken immune cells. Two License Professionnelle students, Corentin Danna and Sofiane El Ouriachi, helped in most part of these preliminary experiments. A Master 2 student, Manon Chaniel, was involved in the studies using chicken aortic endothelial cells.

In our first publication for this project, we used a combination of genome editing (CRISPR-Cas9), PRRs stimulation, inhibitor studies, and infection with DNA pathogens, to reveal that in chicken macrophages, the cGAS/STING pathway is essential not only for the production of type-I interferons in response to intracellular DNA stimulation, but also for regulation of macrophage effector functions including the expression of MHC-II and co-stimulatory molecules. In the context of fowlpox, an avian DNA virus infection, the cGAS/STING pathway was found to be responsible for type-I interferon production and MHC-II transcription. The sensing of fowlpox virus DNA is therefore essential for mounting an anti-viral response in chicken cells and for regulation of a specific set of macrophage effector functions (**Publication 47**).

This collaboration is in line with my research interests and skills, and within the research scope of the UMR ISP. A project that uses information acquired from the MICHIC project, using techniques and rationale developed in other projects. With this collaboration, I strengthen my expertise in macrophages and innate-immune signaling in chickens, increasing my visibility towards other collaborators and creating a niche in a competitive field.

3.16. Avian-beta defensins and chicken innate immunity

I am in a close relationship with Dr. Anne-Christine Lalmanach (now at the team SPVB, UMR ISP). Her former PhD student, Geoffrey Bailleul, was under my co-supervision. Dr. Lalmanach develops research on the antimicrobial activities of avian cationic peptide β -defensins (notably AvBD2 and AvBD7). Defensins are important host defense antimicrobial peptides of animal's innate immune system (Zhao and Lu, 2014, Jarczak et al., 2013, Jager et al., 2012, Hazlett and Wu, 2011). AvBD7 isolated from the chicken bone marrow, possess large antibacterial spectrum and strong resistance to proteolysis. Therefore, the therapeutic potential of AvBD7 to fight threatening bacterial pathogens resistant to multiple conventional antibiotics was assessed in this collaborative project.

For such, we utilized a mouse model of systemic lethal salmonellosis induced by a multidrug-resistant strain of *Salmonella*. As a first approach, fluorescence labeling of AvBD7 allowed to track its systemic distribution after intraperitoneal injection in mice using whole body live imaging. It was associated to peritoneal cells and to deeper organs such as the liver. In the next step, the use of labeled AvBD7 allowed to observe its interaction with murine macrophages in culture. After incubation, it was able to penetrate inside the cells through an endocytosis-like mechanism. Furthermore, natural AvBD7 contributed to the control of intracellular multiplication of a multidrug resistant *Salmonella* strain, after incubation with infected macrophages. Finally, administration in a model of systemic lethal *Salmonella* infection in mice led to significant improvement of mouse survival, consistently with significant reduction of the liver bacterial load. In conclusion, these results revealed a hitherto unknown intracellular antibacterial effect of AvBD7 in *Salmonella* target cells, thus supporting AvBD7 as a candidate of interest for the treatment of infectious diseases caused by multidrug-resistant pathogenic Enterobacteriaceae. I was co-first author in this manuscript (**Publication 41**). We are currently writing a review together with Dutch collaborators on avian defensins and innate immunity in birds. Other collaborative projects on the characterization of AvBDs receptors and cell signaling in chicken macrophages are ongoing, together with a collaboration in the project CAS DAR Chick'Tip.

3.17. Projects CAS DAR (Compte d'Affectation Spéciale « Développement Agricole et Rural »)

In a collaboration with the UMR BOA (Biologie des oiseaux et aviculture, Centre INRAE Val de Loire), the ITEIPMAI (Institut Technique Interprofessionnel des Plantes à Parfum, Médicinales et Aromatique, Chemillé-en-Anjou) and the ITAVI (Institut Technique de l'Aviculture) we got funding for a project under the call *CAS DAR Recherche Technologique* (2017-2020) entitled MEXAVI « “Développement d’une méthodologie éprouvée permettant d’évaluer la capacité des extraits végétaux à renforcer les défenses naturelles des volailles, depuis la sélection des extraits jusqu’à la mesure de l’efficacité biologique. ».

The use of plant extracts to strengthen the immune system and to limit negative consequences of stress is experiencing a growing interest in the poultry industry. However, the development of their use as an additive is hampered in part by the lack of robust and reproducible references related to the complexity of the additives (variability, complex composition) and to the lack of good stress markers or models (reliability, sensitivity). The project aimed to develop and test a reference methodology integrating different stages: the criteria to select the extracts, the assessment of its stability and safety, and its ability to strengthen poultry’s immune system.

I collaborated mostly on the evaluation of potential cytotoxic and/or immune-stimulating effects of selected plant extracts through several *in vitro/ex vivo* methodologies using chicken cell lines (pulmonary, hepatic, macrophages) or blood leukocytes. Preliminary data on 4 extracts (*Mellissa*, *Ginseng*, *Echinacea* and *Nigella*) were promising. They were acquired with the help of a License Professionnelle student (Laurine Allimonnier). We had 2 potential safe and immune-stimulant extracts with strong immune-stimulant properties. I was a work package leader (Action 2). It is a great opportunity to work in collaboration with technical institutes, in close relationship with the French poultry industry. An article is current under preparation.

The intense selection of production performance leads to more efficient animals that are also more demanding and less robust to the disturbances in their environment, with negative consequences on health and welfare. The “start-up” phase, crucial for the future of animals, has become particularly difficult to manage, which reinforces the importance of the quality of the chick. Technicians evaluate the latter using visual assessment indicators that remain subjective and do not reflect the multiple components of this quality (e.g. morphological, physiological, and immunological). Improving the robustness of animals at young age therefore requires the development of new indicators and biomarkers of chick quality. In a collaboration with the UMR BOA and the SYSAAF (Le Syndicat des Sélectionneurs Avicoles et Aquacoles Français), the Chick'Tip (« Un monitoring précoce de la qualité des poussins pour une production avicole plus durable ») project aimed to combine high throughput phenotyping and integrated data analysis methods to revisit the measurement of chick quality and propose tools (antibodies, recombinant proteins, cell lines) to be shared by the actors of breeding, hatching and feeding in the poultry industry. The UMR ISP and myself worked on the Actions 2 and 3, to identify hematological and immune-markers under homeostasis that could be useful to assess chick’s robustness and quality. Articles on the data will be published soon.

3.18. Defining a robust methodology to purify heterophils and identify specific markers as tools to study chicken leukocytes

Heterophils, avian orthologues of mammalian neutrophils, are first line leukocytes believed to be involved in the quick recognition and elimination of poultry pathogens. Techniques for their purification and identification (phenotyping) are still poorly reproducible and unspecific. In a project financed by the Département MICA (2017-2018), I proposed to develop new techniques to purify heterophils and identify specific markers for the generation of antibodies for cell phenotyping, which would allow important progress on the comprehension of acute infectious diseases and chicken leukocyte biology.

Together with the CIRE facility (Chirurgie et Imagerie pour la Recherche et l'Enseignement, UMR PRC, Centre INRAE Val de Loire), we have set up a challenging technique to purify membranes from highly viable blood heterophils following flow cytometry cell sorting (Plateforme Imagerie et Infectiologie - IMI, UMR ISP). With the purified membranes, we performed mass spectrometry analysis to build a panel of proteins present in the surface of heterophils. With this information in hands, we will proceed to comparisons with chicken and mammalian proteomics data banks to build a panel of potential specific proteins present in the surface of heterophils. The aim is to generate antibodies against protein candidates to study heterophils, for the first time, in a highly specific fashion. Moreover, we developed 4 antibodies targeting the highly granulocyte-dominant chemokine receptor CXCR1. Although CXCR1 revealed to be not specific to heterophils in chickens (which was quite unexpected), we now have complimentary antibodies to identify these populations using different immunolabeling techniques (e.g. IHC, IF, flow cytometry).

This important project allowed me, for the first time, to use dedicated money to study a key leukocyte population in chickens. Together with macrophages, heterophils are involved in innate defenses in different diseases of poultry. Very few groups work with these cells abroad. The cross talk between heterophils and macrophages in mucosal surfaces are of interest to the avian immunology community and may bring important insights on the innate immune response within the mucosa, a theme of major interest to the INRAE Departments SA and MICA. I still need time to finish some analyses, but it is a bit obvious that my research focus may start to get centered in these 2 cell populations in chickens, under homeostasis and disease. Also, both cells may play important roles on vaccination, immune stimulation approaches and chicken robustness, key research interests of the poultry industry nowadays. An article is currently under preparation.

3.19. INTEGRITY: “Intestinal barrier integrity and opportunistic infections”

In 2017 we assembled a consortium comprising specialists in avian immunology and infectious diseases at the UMR ISP (Teams PCA and AIM – Françoise Bussi re and Sonia Lamand ), experimental facilities and germ-free animals (PFIE, INRA), nutrition and microbiota (Team AliSE, UMR BOA – Agn s Narcy), and biochemistry (CEPR, INSERM – Giles Lalmanach). The project entitled INTEGRITY: “Intestinal barrier integrity and opportunistic infections” was funded by the *R gion Centre Val de Loire* (2017-2021 – extended contract due to the COVID-19 pandemics), with myself as the project coordinator.

Improved growth performance in poultry production was achieved through genetic selection and feed optimization, which researchers now believe was done at the expense of animal health, with increased incidence of infectious diseases (Hartcher and Lum, 2020, Sakkas et al., 2018, Dennis et al., 2004). Coccidiosis is an intestinal disease caused by the parasite genre *Eimeria* (**Figure 14**), which is very common in poultry farming and is sometimes associated with the appearance of opportunistic diseases (Kovacs et al., 2019, Lillehoj and Lillehoj, 2000). In poultry, the gut microbiota (GM) is critical for the maintenance of intestinal homeostasis, whole body metabolism, immune system maturation, preservation of barrier integrity and pathogen resistance (**Figure 15**). Moreover, current poultry production systems have chicks hatched in very clean environments, where GM development is minimized by the absence of contact to the hens, together with intensive egg surface cleaning and disinfection. Therefore, the establishment of a well-balanced GM in early life is believed to be hampered, with negative consequences to poultry’s health in later life (Yadav and Jha, 2019, Willson et al., 2018, Kogut, 2013). The literature shows that the lesions created during *Eimeria* infection are strongly dependent on the presence of the intestinal microbiota. Therefore, there is a close relationship between the host, the parasite and this microbiota (composed by commensal bacteria and opportunistic pathogens). INTEGRITY aims to study in details the relative importance of these different components in the inflammatory response, in the appearance of intestinal lesions and in the spread of

opportunistic pathogenic bacteria such as *Escherichia coli*. Diet, in addition to its nutrient intake, plays a major role in the health and tightness of the intestinal barrier by acting on the composition of the microbiota, on the leukocyte maturation and on the integrity of the epithelium.

We are also using an alternative diet as a strategy to improve digestive health by promoting the maintenance of intestinal homeostasis and limiting the incidence of parasitic infection. This integrated and innovative approach will provide a better understanding of the pathophysiology of intestinal infections and the origin of some opportunistic infections of poultry.

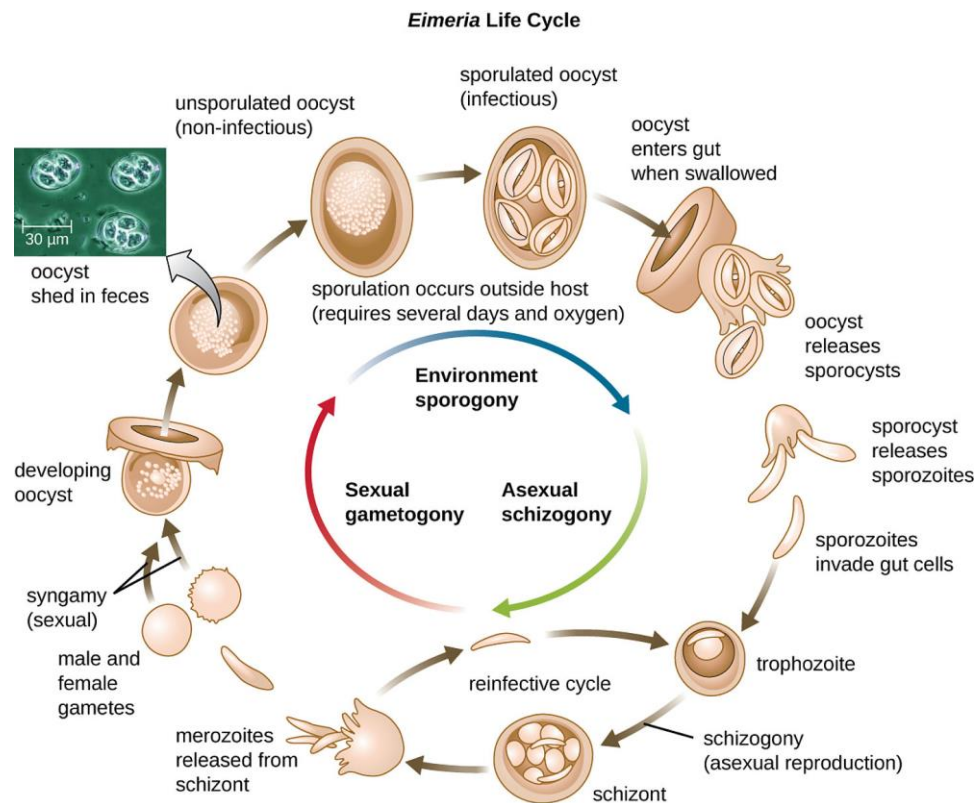


Figure 14. The *Eimeria* life cycle. *Eimeria* cause coccidiosis in chickens and other avian species. Source: CNX OpenStax.

In our more recent publications related to INTEGRITY, we described how we set up a model of germ-free broilers and started the first characterization of the dialogue microbiota-*Eimeria* in chickens. Germ-free chickens are mainly generated from specific-pathogen-free (SPF) experimental lines, which are poorly representative of commercial chicken lines. The method we proposed allowed the production of germ-free chickens from the fast growing broiler line Ross PM3, commonly used by the poultry industry (**Publication 43**). Eggs were quickly collected after laying at a broiler breeder farm. They underwent a strict decontamination process from the collection to the introduction in a sterile egg hatching isolator. The chicks have been hatched and kept in these sterile isolators during the period necessary to control their sterility. Originally developed for an experimental SPF white leghorn line, the present protocol has been adapted not only to the Ross PM3 broiler line but also to quails. It therefore represents a robust and readily adaptable procedure to other poultry species and nesting birds of economic, biological or ecological relevance.

With this animal model in hands, the next objective was to study the impact of the intestinal microbiota on *Eimeria* infection.

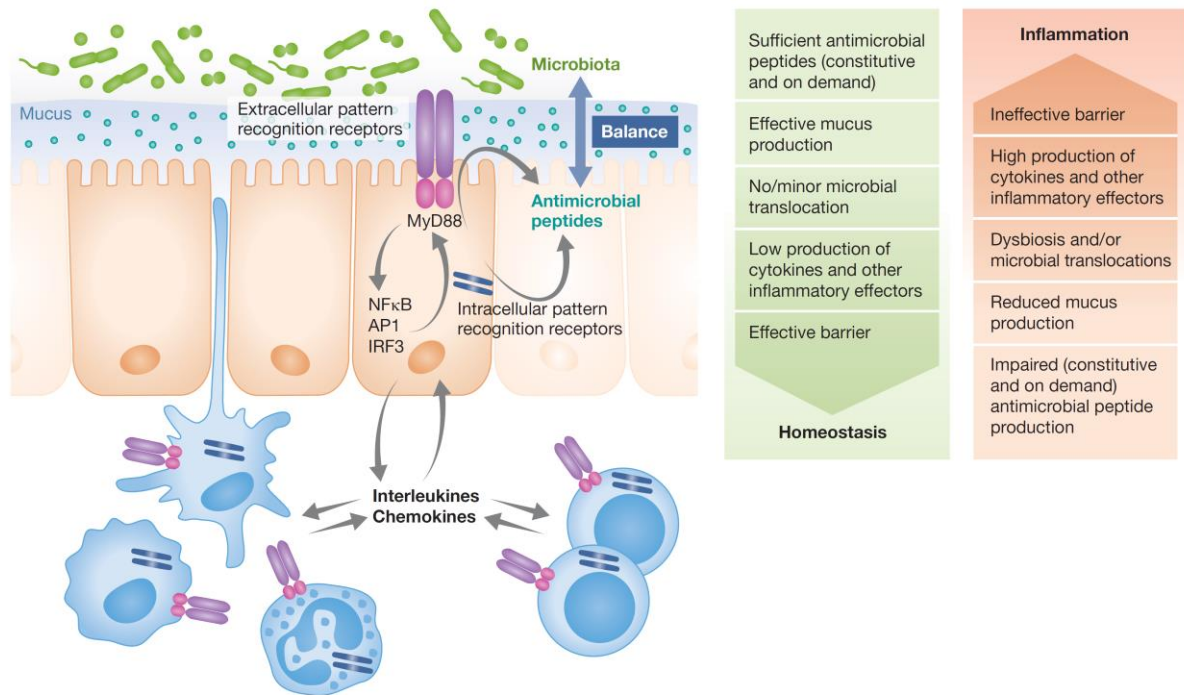


Figure 15. Figure 1. The relationship between resident microbiota and epithelial barrier functions is characterized by a delicate homeostasis. Maturated epithelial cells provide not only a physical shield against the luminal content; they also generate potent biological effectors that help to control intestinal microbiota and keep the epithelial adjacent mucus barrier quite sterile. Among these, various AMPs sourced from all epithelia are crucial in maintaining a beneficial homeostasis in the gut. AMPs are in part constitutively expressed but can also be induced by PRR-activated signalling cascades after stimulation with microbial patterns (Ostaff et al., 2013).

We observed that germ-free chickens presented significantly lower oocyst load in caecal contents when compared to conventional chickens. Histological analysis revealed the presence of significantly less first- and second-generation schizonts in germ-free chickens compared to conventional chickens. We believe these differences in parasite load might result from an initial reduction of the excystation efficiency of the parasite in the gut of germ-free chickens. However, as bile salts involved in the excystation step led to an even higher excystation efficiency in germ-free compared to conventional chickens, this result could not explain the difference in parasite load. Interestingly, when we shunted the excystation step *in vivo* by infecting chickens with sporozoites (using the cloacal route of inoculation), parasite invasion was similar in germ-free and in conventional chickens but still resulted in significantly lower parasite load in germ-free chickens later during infection. Overall, these data highlighted that the absence of intestinal microbiota alters *E. tenella* replication (**Publication 46**). Strategies to modulate the microbiota and/or its metabolites could therefore be an alternative approach to limit the negative impact of coccidiosis in poultry.

Finally, with our colleagues from the CEPR/INSERM, we assessed whether infection by *E. tenella* could alter the intestinal proteolytic balance associated to a modified expression of chicken cysteine proteases. Using germ-free and conventional chickens infected with *E. tenella*, we observed that the basal caecal peptidase activity primarily relies on host proteases rather than proteases from the microbiota. The overall enhanced activity of cysteine cathepsins, as detected after parasitic infection, was attributed to an increase in proteolytically mature avian cathepsin L (CatL), thus supporting that the expression level of host CatL obeys a post-translational regulation associated to *E. tenella* infection. Moreover, since CatL displays regulatory

functions through the inactivation of β -defensins, we hypothesize that avian CatL may participate to the modulation of the inflammatory response occurring during coccidiosis (**article under review**).

This ambitious project will bring new insights on the relevance of improving gut health to prevent intestinal diseases of poultry. We are now able to have germ-free fast-growing chickens in isolators, the coccidiosis infection was set up in these animals and the opportunity to lead a consortium with outstanding collaborators will help solidify my research leadership in the field of poultry mucosal immunology. The project INTEGRITY, for me, is a bridge between poultry sciences and avian immunology. I will be able to connect different actors of poultry research while contributing to the development of tools for the study of avian mucosal immunology together with the team AIM (led by Dr. Sonia Lamandé).

3.20. Other collaborations

With the Team DOVE (UMR BOA), we have been in close contact since 2015 in order to develop a project on innate immune defenses of the avian egg. Two ANR projects were submitted and rejected during the period. However, preliminary experiments now revealed novel and interesting data on how receptors of the innate immunity (e.g. Toll-like receptors), which are able to recognize different pathogens, are differentially modulated during egg embryonic development. I supported the invitation of a Canadian researcher, Prof. Maxwell Hincke, through the *Le Studium* initiative (Université d'Orléans). Prof. Hincke is a specialist of the eggshell. A collaborative review on egg innate immune defenses was recently published (**Publication 39**), together with a book chapter in one of the most relevant books in veterinary immunology: *Avian Immunology (Books and Book chapters 3)*. We are looking forward for further developments on this regard. Still on avian immunology, I have been working with Dr. Veronica Risco-Castillo (ENVA, France) on the characterization of leukocyte populations involved in the chicken and turkey immune response to *Aspergillus* (**Publication 45**).

Finally, I have been collaborating with past colleagues from Brazil, Rafael Marques (CNPq), Gustavo Menezes (UFMG) and Daniel Mansur (UFSC), on activities involving innate immunity mechanisms of defense and cell signaling pathways. These collaborations are minor but important. They involve some few experiments on both sides and exchanges on techniques and protocols. It is important to keep up to date on the human and mouse immunology literature, notably on neutrophils and macrophages, my main expertise.

3.21. Latest updates and Perspectives

Our team at the UMR ISP, "Infection et Immunité Innée chez les Monogastriques" (3IMo), led by Sascha Trapp, was created in January 2019 by merging the team "Immunologie Mucosale Porcine" (IMP), led by Ignacio Caballero, and "Pathologie et Immunologie Aviaire" (PIA), led by Sascha Trapp. I participated in the creation of 3IMo in a scientific effort to put together researchers interested in virology and immunopathology in pigs and birds (both monogastric livestock, together with rabbits). This new structure allowed us to develop work in line with our ambitions, in a hierarchical system functioning in the basis of Principal Investigators (PIs).

Research by 3IMo addresses various aspects of the interplay of avian or porcine viruses with their respective monogastric livestock hosts. Specifically, we seek to elucidate how the pathogenesis of Avian Influenza and African Swine Fever infections is determined by virus-host interactions on the cellular level and to explore if, and to which extent, immunocompetence and infectious disease resistance in monogastric livestock can be shaped by gut microbiota modifications or by treatments with bioactive compounds.

By merging my current experience and panel of collaborators, with new tools and technical approaches, I may be able to place the study of avian immunology in a higher level. The interest is :1. To dissect

inflammatory events that trigger chicken's immune response and **2**. How the microbiota is linked to effective immune responses at infected mucosal surfaces. It is important to mention that metabolites from conserved metabolic pathways (e.g. TCA cycle) and from the microbiota are hot topics. They are critically associated to nutrition and/or anti-inflammation, two topics of major interest to the poultry industry. Attempting to control or influence microbial colonization patterns of the young chicken's gut, including during embryonic development, to promote health and productivity has become a focus in modern poultry production. Birds occupy the same habitats as mammals, have similar ranges of longevity and body mass, and face similar pathogen challenges, yet birds have a different repertoire of organs, cells, molecules and genes of the immune system compared to mammals. It is increasingly evident that the immune system of avian species is very different from those of model mammalian species. Untested extrapolation from mammalian systems cannot provide the quality of knowledge that is required for understanding microbiota-host-pathogen relationships. We are still some way from a clear understanding of the interactions between the GM and immune system components, and the specific interventions that will promote chicken health and productive performance. A project entitled MIMECHICK – “Understanding the contribution of the gut microbiota and its metabolites to the development and functioning of the innate immune system in the chicken” is currently in the second phase of the ANR JCJC 2021. Results are expected in July 2021.

Briefly, MIMECHICK is a refined follow up to INTEGRITY, where we aim to generate comprehensive knowledge on the cellular and molecular mechanisms underlying the contribution of the GM to innate immune system development and functioning in the chicken, including during avian influenza infection. The main expected results are: **(1)** Define which/where innate immune leukocytes are more susceptible to have their development and effector functions modulated by the GM and its metabolites; **(2)** Determine the identity and concentrations of GM-derived metabolites in different organs and unveil their immunomodulating properties in *bona fide* chicken cell lines and purified leukocytes; **(3)** Uncover the consequences of dysbiosis caused by avian influenza infection in young chickens, with detailed information on cellular and molecular immunological signatures likely to be altered in various organs during growth; and **(4)** Provide immunological (cells, mediators) and physiopathological (viral replication, mucosal damage) insights on how the microbiota is essential to determine resistance to avian influenza infection along the gut-lung axis.

Overall, data arising from these projects and the established collaborations will have direct impact on the management of chicken robustness in face of infectious diseases in poultry farms, which will therefore strengthen mine and the UMR ISP relationship with the ANSES, veterinary schools and poultry farmers within the next years. My wish is to continue promoting research on avian immunology, a rare topic in other French research structures. I am frequently being requested to support studies and/or engage in new collaborations within this topic. My passion for science and my will to collaborate will certainly evolve by the moment I acquire the HDR, which will open doors to autonomy, new mentorship activities and to the development of more focused and sophisticated projects.

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5. Synthèse en Français

Ce rapport de recherche présente les aspects le plus importants de ma carrière scientifique, qui comprend la mise en place d'un réseau de collaborations internationales et la publication d'articles d'importance majeure dans les domaines de la pharmacologie, la physiologie, l'immunologie et les maladies infectieuses. Outre mon activité de recherche, j'ai également eu une expérience dans l'enseignement à l'Université Fédéral de Minas Gerais (UFMG) au Brésil. La compréhension des interactions hôte-pathogène, bien comme de la pathogenèse de l'inflammation et de l'infection est cruciale pour la mise au point de nouvelles thérapies pour soulager la souffrance des patients et des animaux. Je pense que toutes ces activités constituent une base solide pour appuyer ma demande d'obtention d'une Habilitation à Diriger des Recherches (HDR).

Résumé des travaux de recherche

L'ischémie intestinale et les lésions de reperfusion

L'ischémie intestinale est relativement commune chez l'homme après un polytraumatisme et réduit considérablement le pronostic vital du patient. En effet, un traumatisme abdominal intestinale entraîne dans certains cas une ischémie suivie d'une reperfusion (I/R), provoquant ainsi des lésions intestinales et pulmonaires sévères. Comprendre le rôle des cytokines pro- et anti-inflammatoires impliquées dans le processus conduisant aux lésions tissulaires, notamment à travers l'activation et l'accumulation des neutrophiles, permettrait de clarifier les mécanismes mis en jeu au cours de l'I/R. Grâce à un modèle chirurgical reproduisant la pathologie chez le rat, nous avons montré que l'IL-1 β endogène induisait une production d'IL-10 qui à son tour protège contre la réponse inflammatoire aiguë systémique et locale suite à l'I/R (Souza et al, 2003). La démonstration qu'une cytokine pro-inflammatoire puisse moduler la production de cytokines anti-inflammatoires dans un modèle d'inflammation aiguë et chronique, était à l'époque de la plus haute importance et a débouché sur plusieurs projets dans de multiples modèles inflammatoires au sein de notre équipe. Cette première expérience avec le modèle expérimental d'I/R a conduit à la collaboration avec le Dr Wothan Tavares De Lima (USP, Brésil) et avec le groupe du Dr Bernhard Ryffel (CNRS Orléans). Ces collaborations ont permis de démontrer que l'inflammation pulmonaire et intestinale suite à une I/R dépend de la voie de signalisation TLR/MyD88, et que la production de TNF- α et d'IL-1 β est à l'origine du recrutement des neutrophiles dans les poumons et l'intestin, de la fuite capillaire et de la bactériémie (Freitas et al, 2010). Cette prolifique collaboration a également permis la publication de travaux portant sur la signalisation TLR2 / 4 dans un modèle inflammation pulmonaire locale et systémique (Soares et al. 2010). Mon expérience de recherche avec le modèle d'I/R chez le rat et la souris a largement contribué à ma formation en pathologie et en expérimentation animale.

La fibrose pulmonaire

La fibrose pulmonaire peut être causée par une inflammation chronique ou par un *cross-talk* perturbé entre cellules épithéliales et fibroblastes, ce qui peut se produire en absence d'inflammation. Certaines études ont mis en évidence un rôle important des neutrophiles dans le processus inflammatoire qui précède la fibrose pulmonaire induite par la bléomycine, un agent de chimiothérapie utilisé chez l'humain pour reproduire expérimentalement la maladie chez la souris. Nous avons décrit les effets protecteurs du DF2162 (Dompé Pharmaceuticals, Italie), un antagoniste à longue durée d'action du récepteur de chimiokine CXCR2 (important

pour le recrutement des neutrophiles vers les sites inflammatoires), sur la progression de la fibrose pulmonaire induite par la bléomycine chez la souris. La neutralisation de CXCR2 entraîne une réduction de la migration des neutrophiles dans les voies aériennes, la réduction des cytokines pro-fibrotiques et une diminution globale de la pathologie (Russo et al, 2009). Dans le prolongement de ce travail, Russo et al. (2011) ont montré que la fibrose pulmonaire et la létalité induite par la bléomycine sont nettement atténuées chez les souris déficientes pour PI3K γ . PI3K γ est une molécule centrale dans la signalisation de nombreuses fonctions cellulaires principalement chez les cellules immunitaires, et plus particulièrement chez les neutrophiles. En l'absence de PI3K γ , l'influx et l'activation leucocytaire, la transcription des marqueurs fibrotiques et l'angiogenèse pulmonaire sont réduits. A travers ce second projet, j'ai eu l'opportunité de collaborer avec des compagnies pharmaceutiques et des groupes de recherche fondamentale, mettant à profit ma rigueur scientifique et mes facultés de communication.

Les produits naturels et les espèces réactives de l'oxygène

Au Brésil, j'ai eu l'occasion de collaborer avec le Professeur Virginia Lemos (UFMG), une physiologiste expérimentée dans l'étude des maladies cardio-vasculaires. A cette époque, le professeur Lemos s'intéressait à la Diocleïn, un flavonoïde isolé à partir des racines de *Dioclea grandiflora*. Elle a notamment mis en évidence les effets vasodilatateurs et hypotenseurs de la Diocleïn, mais ses effets anti-inflammatoires étaient inconnus. De par leurs propriétés antioxydantes, les flavonoïdes peuvent retarder l'apparition de l'athérogènes en réduisant la libération de médiateurs inflammatoires et ainsi réduire les événements thrombotiques. C'est pourquoi notre groupe a testé les effets de la Diocleïn dans les cellules inflammatoires. Avec l'aide du Dr Claire Luginier (CNRS, Strasbourg), nous avons démontré les effets inhibiteurs de la Diocleïn sur les phosphodiesterases (PDE) et plus particulièrement sur la PDE de type 4, dont la fonction est de métaboliser l'AMPc dans les cellules immunitaires, conduisant à la production de cytokines, de chimiokines et d'espèces réactives de l'oxygène. Nous avons testé la Diocleïn (pureté 99% / HPLC) sur des neutrophiles et des macrophages murins purifiés, et montré que la Diocleïn ou ses dérivés peuvent être utilisés dans le développement de nouveaux médicaments anti-inflammatoires (Guabiraba et al. 2010). Le double mécanisme d'action de la Diocleïn - inhibition de la PDE4 et de la production d'espèces réactives de l'oxygène - supporte la notion que les médicaments qui agissent sur des cibles multiples peuvent être plus efficaces pour traiter l'inflammation. Pendant ce temps, j'ai obtenu un diplôme de spécialisation en Pathologie Vasculaire au Département de Physiologie et Biophysique à l'UFMG.

L'angiogenèse inflammatoire

L'angiogenèse est un processus complexe impliqué dans le développement des vaisseaux sanguins et dans les réactions inflammatoires. Après avoir travaillé avec les modèles d'I/R expérimentales et acquis une bonne expérience *in vivo* avec la mise en place du modèle de fibrose pulmonaire au laboratoire du Professeur Mauro Teixeira (UFMG), j'ai été invité à faire partie du groupe d'étude de l'angiogenèse au cours de mon Master (2005-2007). Mon premier projet était d'identifier le rôle des chimiokines CCL3/MIP-1 α et CCL5/RANTES (impliqués dans le recrutement des monocytes/lymphocytes vers les sites inflammatoires) au cours du développement de la néo-vascularisation. L'étude a révélé un effet anti-angiogénique *in vivo* inattendu pour ces deux chimiokines. En effet, l'activation des récepteurs de CCL5/RANTES (CCR1 et CCR5) inhibe l'angiogenèse inflammatoire chez la souris (Barcelos et al, 2009). L'intérêt pour les récepteurs cannabinoïdes et le système endocannabinoïde est encore un domaine en pleine expansion, où les composants du système immunitaire et nerveux sont étroitement liés. Sanofi-Aventis a développé

Rimonabant®, un antagoniste du récepteur cannabinoïde CB1, pour le traitement de l'obésité, qui présente également des propriétés anti-inflammatoires dans différents modèles expérimentaux pour diverses maladies. Au laboratoire, nous avons étudié les effets du Rimonabant® et d'un antagoniste synthétique des récepteurs CB2 (SR144528) sur le système endocannabinoïde au cours de l'angiogenèse inflammatoire. En utilisant des approches immunologiques et physiopathologiques, nous avons montré que le blocage des récepteurs cannabinoïdes permet de réduire l'inflammation et l'angiogénèse. La modulation des cytokines, des chimiokines et des endocannabinoïdes peuvent contribuer à expliquer cette réponse. L'activité d'agoniste partiel / agoniste inverse et la désensibilisation des récepteurs peuvent également expliquer les effets antagonistes sur les récepteurs cannabinoïdes (Guabiraba et al., 2013). Après avoir travaillé avec trois différents modèles expérimentaux d'inflammation aiguë et chronique, et disséqué les mécanismes cellulaires et moléculaires impliqués dans les processus de chaque pathologie, j'ai pu consolider les qualités importantes du chercheur: l'organisation, la collaboration et la rigueur scientifique.

Les chimiokines et l'infection par *Schistosoma mansoni*

La schistosomiase est l'une des infections à helminthes les plus répandues dans le monde, transmise par les vers du genre *Schistosoma*. Chez les personnes infectées, on observe une réponse immunitaire exacerbée et une inflammation granulomateuse avec dépôt de collagène en réponse à la ponte des œufs (dans le foie, dans le cas de *Schistosoma mansoni*). Nous avons démontré une corrélation positive entre les concentrations plasmatiques élevées de CCL3/MIP-1 α et la probabilité de présentation de la schistosomiase hépatosplénique sévère chez l'homme. Nous avons également montré dans un modèle murin de l'infection qu'une déficience en CCL3 réduisait la morbidité (Souza et al. 2005). La manipulation d'échantillons humains a nécessité un strict respect des règles de biosécurité et de bioéthiques. Juste après mon Master, je me suis engagé dans un autre projet sur la schistosomiase expérimentale, impliquant le récepteur de chimiokines CCR5. Ce projet a été soutenu par une importante subvention (PNUD / Banque mondiale / OMS Programme spécial de recherche et de formation sur les maladies tropicales) et a débouché sur une accumulation d'observations importantes sur le rôle des chimiokines dans la sévérité de la schistosomiase. Plus précisément, nous avons montré que l'absence de CCR5 entraînait une réduction du recrutement des cellules Foxp3+ dans les lésions granulomateuses, ainsi qu'une élévation des niveaux de CCL3/MIP-1 α , et l'augmentation de l'infiltration de macrophages dans le foie, ce qui suggère un rôle modulateur de CCR5 au cours de l'infection à *S. mansoni* (Souza et al, 2011). Les techniques classiques de parasitologie telles que l'amplification du parasite *in vivo* ou *in vitro*, le suivi de l'infection chez l'animal et l'étude de la physiopathologie hépatique (par échographie, histologie ou par l'isolement de granulomes) m'ont été fortement utiles par la suite. En effet, c'est en 2006 qu'ont débuté mes travaux en virologie, d'abord sur le virus de la Grippe A (Influenza), puis sur le virus de la Dengue, à nouveau dans le groupe du Pr Teixeira.

L'infection par le virus de la grippe A et l'inflammation pulmonaire

Le virus influenza A provoqué chaque année des épidémies affectant des millions de personnes et causant entre 250.000 et 500.000 décès à travers le monde. Les stratégies visant à réduire la réponse inflammatoire, sans affecter l'élimination du pathogène pourraient réduire les hospitalisations, la mortalité, ainsi que les pertes économiques liées à la maladie. Le modèle expérimental de l'infection par l'Influenza A a été mis en place dans le groupe du Pr Teixeira en 2006 en collaboration avec le professeur Ricardo T. Gazzinelli (UFMG). Nos études ont clairement montré le rôle du PAF (facteur d'activation plaquettaire) dans l'activation de l'inflammation au cours de l'infection par le virus influenza A. Le PAF est un phospholipide impliqué dans

la transmigration des leucocytes, la modification de la perméabilité vasculaire, l'hypotension, la production de diverses cytokines et des lésions pulmonaires. Nos travaux ont montré que les souris déficientes pour le récepteur du PAF présentent une infiltration de neutrophiles et de macrophages réduite dans les voies respiratoires et une diminution des dommages pulmonaires. L'absence du récepteur du PAF (PAFR) permet de renforcer la réponse adaptative, et plus particulièrement la réponse humorale, et d'éliminer plus efficacement le virus. Le traitement par des antagonistes du PAFR diminue la mortalité chez la souris (Garcia et al., 2010). A la même époque, le groupe du Pr Teixeira a mis en place un nouveau modèle d'infection par le virus de la Dengue chez la souris. Ce projet a rapidement abouti à une publication importante (PNAS) et ce modèle s'est avéré être très avantageux pour étudier cette infection et les réponses immunitaires associées. Ma participation à ce projet fut d'importance majeure pour ma carrière et a défini mon profil scientifique actuel.

Infection par le virus dengue

La fièvre de la dengue et le syndrome de choc de la dengue (DF / DSS) sont des infections virales transmises par les moustiques. On distingue quatre sérotypes du virus responsable de la Dengue (DENV 1-4). On dénombre environ 50-100 millions de cas de dengue et 20.000 décès par an principalement dans les régions tropicales et subtropicales du monde. Le DSS est défini comme une fièvre hémorragique, associée à une thrombocytopénie, une hémococoncentration ou d'autres signes de fuite plasmatique, et à une "tempête" de cytokines. Il n'existe pas de médicaments approuvés pour le traitement de l'infection par la Dengue. Dr George Ignatiev ("Vector" Institut, Koltsovo, Russie) a commencé sa collaboration avec le Brésil en donnant 2 souches virales isolées de l'humain et adaptées à la souris de sérotypes DENV-2 et 3. J'ai contribué à la caractérisation *in vitro* du virus (principalement DENV-2) en infectant différents types cellulaires tels les cellules HepG2, les fibroblastes et les cellules dendritiques. Nous avons ainsi caractérisé la production de chimiokines et de cytokines, la cytotoxicité, la réplication virale et l'identité virale (PCR). La présence du virus a pu être confirmée par des marquages immunohistochimiques et immunofluorescents des protéines NS3 et E, respectivement, dans les foies ou dans des cellules HepG2 (Fagundes et al. 2011, Guabiraba et al, 2013). De récentes études cliniques ont décrit une corrélation entre les formes sévères de la dengue et les taux sanguins de chimiokines de type CC. J'ai donc commencé à étudier le rôle du récepteur CCR5 dans un modèle expérimental d'infection par LE DENV-2. L'utilisation *in vitro* et *in vivo* d'antagonistes du récepteurs CCR5 (Met-RANTES ou Maraviroc®) ont montré une réduction de la charge virale et une protection des souris. La même chose a été observée chez des souris déficientes en CCR5. Ce même récepteur participe à l'entrée du HIV dans les cellules T et au trafic des leucocytes au cours de l'infection par le virus West Nile. Nous avons démontré *in vitro* que CCR5 était important pour l'entrée du virus de la dengue et pour sa réplication. Nous avons obtenu un soutien de Pfizer (Royaume-Uni) pour tester Maraviroc et d'autres antagonistes de CCR5 dans notre modèle avant la publication (Marques and Guabiraba et al. 2015). C'est à cette période que je suis venu travailler en France pendant une année complète au sein du laboratoire du Dr Bernhard Ryffel (CNRS, Orléans). L'accès à un réseau scientifique important a rendu cet échange très prolifique. J'ai notamment étudié le rôle des récepteurs CCR1, CCR2 et CCR4 au cours de l'infection par DENV-2 chez la souris. Il semblerait que ces récepteurs ne jouent pas un rôle essentiel dans l'élimination du virus, mais contribuaient plutôt à l'excès d'inflammation et aux manifestations de la maladie (Guabiraba et al, 2010). Un second projet en collaboration avec le Dr François Trottein (Institut Pasteur, Lille) a consisté en l'étude des cellules iNKT dans l'infection par la dengue. Ces cellules, bien que peu nombreuses dans l'organisme humain, jouent un rôle clé dans les réponses immunes innées. La coordination du projet avec le Dr Joëlle Renneson (Institut Pasteur, Lille), a permis de montrer un rôle critique des cellules iNKT dans le développement de la maladie et la mortalité

associées à l'infection par DENV-2. Ces cellules s'accumulent dans les tissus (foie et rate), et produisent en continu des médiateurs pro-inflammatoires contribuant aux dommages tissulaires fatals (Renneson and Guabiraba *et al.* 2011). Ces travaux ont constitué la base de mon travail de thèse.

Je me suis également intéressé au rôle d'une nouvelle classe de cytokines (Th17) dans l'infection par DENV-2. IL-22 et IL-17 sont des cytokines ayant des fonctions généralement distinctes. Alors que l'IL-22 est globalement protectrice, IL-17 contribue à la production de chimiokines et l'activation des leucocytes dans différentes maladies chez l'homme et la souris. Il a été montré que les cellules iNKT peuvent produire de l'IL-17. Nous avons ainsi montré que l'IL-22 était surtout impliquée dans la survie des hépatocytes lors de l'infection, en maintenant l'homéostasie tissulaire et en réduisant la mort cellulaire et la production de cytokines. IL-22 n'est pas impliquée dans la réplication virale et sa signalisation n'est pas essentielle pour la clairance virale. A l'inverse, l'IL-17 est pro-inflammatoire et contribue fortement à l'activation des lymphocytes NKT et à la cytotoxicité hépatique (Guabiraba *et al.* 2013). Suite à ces publications, 2 importantes subventions ont été obtenues (INSERM / CNPq et INCT / CNPQ) pour continuer le projet. Au cours de mes 4 années de doctorat, j'ai supervisé 2 étudiants de master qui ont activement participé au projet CCR5.

Mon expérience dans l'enseignement à l'Université Fédérale de Minas Gerais (Brésil)

En 2008, j'ai été sélectionné par concours pour remplacer un professeur en Physiologie Humaine et Biophysique à l'Université de Minas Gerais pour une période de 6 mois (UFMG, Brésil). Cette expérience dans l'enseignement m'a particulièrement plu et m'a beaucoup enrichi aussi bien scientifiquement qu'humainement. Pendant cette période, j'ai enseigné la Biophysique et la Physiologie Humaine avec en moyenne 20 heures de cours par semaine (soit un total de 400 heures). Cette expérience m'a permis de développer de nombreuses compétences essentielles dans l'enseignement, en particulier en matière de préparation de cours, d'organisation et de gestion du temps. Les professeurs du département m'ayant donné un certain degré de liberté, j'ai pu préparer les cours théoriques d'une manière très personnelle. J'ai adapté chacun de mes cours en fonction des connaissances préliminaires en biologie des étudiants et des objectifs professionnels ciblés (e.g. Licence en Biologie ou en Nutrition). Les séances de travaux pratiques m'ont amené à étudier différents aspects de la physiologie à travers des expériences *in vitro* ou sur animaux vivants. Les séances de TP portaient sur l'étude du système cardio-vasculaire chez le chien et chez le rat, avec des expériences en neurophysiologie sur muscles isolés, ainsi que différentes expériences biochimiques et enzymatiques pour comprendre le métabolisme et les fonctions rénales. A l'issue de ces 6 mois d'enseignement, c'est avec regret que j'ai décidé de ne pas poursuivre le contrat afin de me concentrer sur l'écriture de mon rapport de thèse et de préparer mon voyage en France au début de 2009. A la même époque (entre 2007 et 2010), toujours à l'UFMG, j'ai donné 4 séries de séminaires destinés au grand public afin de discuter de nombreux aspects de la biologie et de la pharmacologie des drogues addictives (comme le cannabis, la cocaïne ou l'héroïne). Au cours de ces séminaires, j'ai utilisé mes connaissances en pharmacologie pour aider le public et les jeunes étudiants à comprendre ce problème social d'une manière beaucoup plus scientifique, en exposant des faits historiques et des mythes sur la consommation de drogues. Au total, j'ai donné 48 heures de séminaires devant un auditoire d'environ 20-30 personnes.

Modele de colite induite par le DSS (dextran sodium sulphate)

Les maladies inflammatoires de l'intestin (MICI), qui comprennent la colite ulcéreuse (CU) et la maladie de Crohn, sont des pathologies chroniques, récurrentes, et provoquées par des états inflammatoires d'origine inconnue. La participation des granulocytes neutrophiles dans la pathogénie des MICI est bien connue, mais

peu d'attention a été accordée au rôle des éosinophiles. Nous avons montré chez la souris que le blocage du recrutement des éosinophiles avec un antagoniste actif de CCL11 (Merck-Serono, Suisse) prévient la maladie clinique, la destruction des tissus, et la mort. (Vieira et al. 2009). J'ai personnellement contribué à l'analyse histologique de l'intestin et à la caractérisation *in vivo* du composé en utilisant la cavité pleurale chez les souris. Pendant mon séjour en France, j'ai eu l'occasion de collaborer avec le Dr Thomas Secher (INSERM Toulouse) sur une étude portant sur le rôle de l'IL-22 dans un modèle murin de colite expérimentale. Nous avons montré que l'IL-22 est essentielle pour la préservation de l'épithélium intestinal au cours de l'inflammation induite par le DSS, et que cette cytokine participe au maintien de l'intégrité de la barrière intestinale en empêchant la translocation bactérienne et l'exacerbation de l'inflammation (données non publiées).

Le rôle de l'IL-33 sur les lymphocytes Th2 et une mucosite induite par chimiothérapie

Juste après la fin de ma thèse, j'ai été recruté pour un Post-Doc de 3 ans avec le Professeur Foo Liew (Glasgow, Royaume-Uni), un immunologiste spécialiste de l'allergie et de l'immunité innée (Février, 2011). Deux projets m'ont été confiés, l'un portant sur l'influence de l'IL-33 sur les micro-ARN (miRNA) dans les cellules Th2 et le second portant sur le rôle de l'axe IL-33/ST2 dans la mucosite induite par l'agent de chimiothérapie Irinotécan. L'IL-33 peut fonctionner à la fois comme une cytokine traditionnelle et comme un facteur de régulation de la transcription des gènes nucléaires. Elle est produite rapidement par les cellules épithéliales lors de stress ou de lésions tissulaires, et est souvent considérée comme une « alarmine ». Au cours de ce post-doc, j'ai pu approfondir mes connaissances en biologie moléculaire et protéomique en étudiant les miRNA dans les cellules primaires Th2. Certains candidats potentiels ont été identifiés. La mucosite est un problème fréquent et grave chez les patients en cours de chimiothérapie anticancéreuse, conduisant à des diarrhées et une perte de poids rapide. De par sa fonction d'alarmine, l'IL-33 était un candidat potentiel dans la mise en place de l'inflammation aiguë de l'intestin induite par le traitement à l'irinotecan (CPT-11). Nous avons d'abord montré que le blocage du récepteur ST2 réduisait la mucosite chez la souris, en agissant sur le recrutement des neutrophiles. En effet, les niveaux en IL-10 sont augmentés chez les souris déficientes en ST2, ce qui contribue à la sous-expression du récepteur CXCR2 à la surface des neutrophiles. Nous cherchons à présent à savoir si le blocage du récepteur ST2 pendant la chimiothérapie du cancer pourrait permettre de soulager les effets secondaires et ainsi améliorer l'efficacité du traitement.

Travaux à l'INRAE et Perspectives

J'ai été recruté en tant que Chargé de Recherche (CRCN) à l'INRAE en Juillet 2013. J'ai officiellement démarré mes activités à l'UMR ISP à Nouzilly en Janvier 2014. L'Unité de Recherche UMR ISP (Infectiologie et Santé Publique) à Nouzilly consacre ses recherches aux maladies infectieuses représentant des menaces pour la santé animale et/ou humaine (le concept « One Health »). Il y avait un intérêt croissant et un ample soutien de la direction (Dr. Nathalie Winter) pour le développement d'études plus mécanistiques sur l'immunologie aviaire. Mon recrutement était directement lié à cet intérêt croissant. L'UMR ISP est composée d'excellentes équipes étudiant différents aspects de la réponse de l'hôte aux maladies de l'élevage, une référence en France. L'équipe PCA (Pathogénie de la Colibacillose Aviaire), à l'époque animée par le Dr Catherine Schouler, avait une expertise en épidémiologie moléculaire et dans la caractérisation des souches d'*Escherichia coli* pathogènes aviaires (APEC), y compris l'analyse de l'adhésion et de l'invasion bactérienne dans les tissus et les cellules de l'hôte. J'ai été recruté dans l'équipe PCA en tant qu'immunopathologiste, avec une expertise dans les modèles *in vivo* et *in vitro* pour l'étude de l'infection et de l'inflammation. J'ai trouvé le sujet extrêmement intéressant, notamment sur la possibilité d'élargir mes horizons dans les mécanismes immunologiques

opérant chez les espèces non-mammifères. C'est une belle occasion de développer un nouveau sujet dans un domaine de recherche souvent négligé, ce qui pourrait signifier moins de concurrence dans le domaine et un tout nouveau réseau de collègues.

Mon objectif initial était d'étudier les médiateurs vasculaires impliqués dans la réponse précoce de l'hôte à l'infection, qui sont étroitement associés aux lésions tissulaires et à la clairance bactérienne, notamment le facteur d'activation des plaquettes (PAF) et l'ornithokinine (OK, l'analogue aviaire de la bradykinine des mammifères). Ces médiateurs vasculaires sont rapidement produits (en moins d'une heure) sur les sites infectés et jouent un rôle dans le développement précoce des réponses inflammatoires locales. L'étude des molécules participant à l'interface sang-tissu lors de l'infection chez les oiseaux reste largement sous-développée et constitue un excellent champ de recherche dans le contexte de l'immunologie aviaire. Ayant déjà travaillé sur les médiateurs lipidiques, l'angiogenèse et les interactions cellule-tissu, mon intérêt pour le rôle du PAF et de l'OK dans le contexte de la colibacillose était justifié. Étant donné que l'équipe PCA s'intéressait aux événements précoces de l'adhésion et de la colonisation des voies respiratoires du poulet par les bactéries, l'étude des médiateurs vasculaires ayant des effets immunomodulateurs pourrait apporter des informations importantes non seulement pour la compréhension de la pathogenèse de la colibacillose chez la volaille mais aussi pour l'interaction hôte-pathogène chez le poulet.

Avec le Dr. Sascha Trapp (à cette époque dans l'équipe PIA, UMR ISP), le Dr. Schouler a établi une collaboration avec le Roslin Institute, UK et la LMU, Allemagne, pour la soumission d'une proposition de subvention (ANIHWA/ERA-NET) en 2014. J'ai donc pu participer à l'élaboration des work-packages afin d'apporter une expertise et d'ouvrir de nouvelles possibilités de recherche. Le projet "MICHIC" : *Understanding mucosal immunology and co-infections in the chicken to drive vaccine strategies* a reçu un financement pour 3 ans (2015-2018). Ce fut une excellente occasion d'échanger des connaissances avec les collaborateurs européens et d'améliorer les compétences sociales et de gestion avec Damien Garrido, doctorant. Nous avons publié 2 articles dans le cadre du projet MICHIC et Damien a collaboré à 2 autres articles de mes projets individuels (PAF et OK). Un autre manuscrit est encore en préparation (GM-CSF et différenciation des macrophages dans le sang).

Fin 2015, le Dr Brian Ferguson (Département de Pathologie, Université de Cambridge, Royaume-Uni) m'a contacté pour une collaboration. Le Dr Ferguson est un spécialiste de la "détection" de l'ADN chez les mammifères. Il s'intéresse particulièrement à la manière dont la détection des acides nucléiques déclenche une réponse immunitaire innée chez différentes espèces, comme le poulet. Cette collaboration s'inscrit dans le cadre de mes intérêts et compétences de recherche, et dans le champ de recherche de l'UMR ISP. Un projet qui utilise les informations acquises dans le cadre du projet MICHIC, en utilisant des techniques et des raisonnements développés dans d'autres projets. Grâce à cette collaboration, je renforce mon expertise dans le domaine des macrophages et de la signalisation immunitaire innée chez les poulets, augmentant ainsi ma visibilité vis-à-vis des autres collaborateurs et créant une niche dans un domaine relativement compétitif.

En 2017, nous avons réuni un consortium comprenant des spécialistes de l'immunologie aviaire et des maladies infectieuses de l'UMR ISP (équipes PCA et AIM - Françoise Bussière et Sonia Lamandé), des installations expérimentales et des animaux axéniques (PFIE, INRA), de la nutrition et du microbiote (équipe AliSE, UMR BOA - Agnès Narcy), et de la biochimie (CEPR, INSERM - Giles Lalmanach). Le projet intitulé « INTEGRITY » : *Intestinal barrier integrity and opportunistic infections* a été financé par la Région Centre Val de Loire (2017-2021 - contrat prolongé en raison de la pandémie au COVID-19), avec moi-même comme coordinateur du projet.

Notre équipe à l'UMR ISP, " Infection et Immunité Innée chez les Monogastriques " (3IMo), dirigée par Sascha Trapp, a été créée en janvier 2019 par la fusion de l'équipe " Immunologie Mucosale Porcine " (IMP), dirigée par Ignacio Caballero, et " Pathologie et Immunologie Aviaire " (PIA), dirigée par Sascha Trapp. J'ai

participé à la création de 3IMo dans un effort scientifique visant à regrouper les chercheurs intéressés par la virologie et l'immunopathologie chez le porc et les poulet (deux animaux de rente monogastriques, ainsi que les lapins). Cette nouvelle structure nous a permis de développer des travaux à la hauteur de nos ambitions, dans un système hiérarchique fonctionnant sur la base de *Principal Investigators* (PIs).

Les recherches menées par 3IMo portent sur divers aspects de l'interaction entre les virus aviaires ou porcins et leurs hôtes monogastriques respectifs. Plus précisément, nous cherchons à élucider la manière dont la pathogenèse des infections par la grippe aviaire et la peste porcine africaine est déterminée par les interactions virus-hôte au niveau cellulaire, et à explorer si, et dans quelle mesure, l'immunocompétence et la résistance aux maladies infectieuses chez les animaux monogastriques peuvent être façonnées par des modifications du microbiote intestinal ou par des traitements avec des composés bioactifs.

Les oiseaux occupent les mêmes habitats que les mammifères, ont une longévité et une masse corporelle similaires et sont confrontés à des pathogènes similaires. Pourtant, les oiseaux ont un répertoire d'organes, de cellules, de molécules et de gènes du système immunitaire différent de celui des mammifères. Il est de plus en plus évident que le système immunitaire des espèces aviaires est très différent de celui des espèces mammifères modèles. L'extrapolation non testée à partir des modèles mammifères ne peut pas fournir la qualité de connaissance nécessaire à la compréhension des relations microbiote-hôte-pathogène chez le poulet. Nous sommes encore loin d'une compréhension claire des interactions entre les composants du microbiote intestinal et du système immunitaire, et des interventions spécifiques qui favoriseront la santé et les performances productives des poulets. Un projet intitulé MIMECHICK - "Comprendre la contribution du microbiote intestinal et de ses métabolites au développement et au fonctionnement du système immunitaire inné chez le poulet" est actuellement dans la deuxième phase de l'ANR JCJC 2021. Les résultats sont attendus pour juillet 2021. En bref, MIMECHICK est un suivi affiné d'INTEGRITY, où nous visons à générer des connaissances complètes sur les mécanismes cellulaires et moléculaires qui sous-tendent la contribution du microbiote intestinal au développement et au fonctionnement du système immunitaire inné chez le poulet, y compris pendant l'infection par la grippe aviaire.

Globalement, les données issues de ces projets et les collaborations établies auront un impact direct sur la gestion de la robustesse des poulets face aux maladies infectieuses dans les élevages avicoles, ce qui renforcera donc mes relations et celles de l'UMR ISP avec l'ANSES, les écoles vétérinaires et les éleveurs de volailles dans les prochaines années. Mon souhait est de continuer à promouvoir la recherche en immunologie aviaire, une thématique rare dans les autres structures de recherche françaises. Je suis fréquemment sollicité pour soutenir des études et/ou engager de nouvelles collaborations dans cette thématique.

Ma passion pour la science et ma volonté de collaborer évolueront certainement au moment de l'acquisition du HDR, qui m'ouvrira les portes de l'autonomie, de nouvelles activités de mentorat et du développement de projets plus ciblés et sophistiqués.